Clinical Study Protocol

NCT Number: NCT04879849

Title: An Open-Label, Phase 1, Dose-Escalation Study to Evaluate the Safety and Preliminary Antitumor Activity of TAK-676 With Pembrolizumab Following Radiation Therapy in the Treatment of Non–Small-Cell Lung Cancer, Triple-Negative Breast Cancer, or Squamous-Cell Carcinoma of the Head and Neck that Has Progressed on Checkpoint Inhibitors

Study Number: TAK-676-1003

Document Version and Date: Amendment 2.0, 18 February 2022

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

A summary of changes to previous protocol versions is appended to the end of the document.

PROTOCOL

An Open-Label, Phase 1, Dose-Escalation Study to Evaluate the Safety and Preliminary Antitumor Activity of TAK-676 With Pembrolizumab Following Radiation Therapy in the Treatment of Non–Small-Cell Lung Cancer, Triple-Negative Breast Cancer, or Squamous-Cell Carcinoma of the Head and Neck that Has Progressed on Checkpoint Inhibitors

TAK-676 With Pembrolizumab Following Radiation Therapy in the Treatment of Non-Small-Cell Lung Cancer, Triple-Negative Breast Cancer, or Squamous-Cell Carcinoma of the Head and Neck

Sponsor:	Takeda Development Center Americas, Inc. (TDC Americas) 95 Hayden Avenue Lexington, MA 02421 Please note: Takeda Development Center Americas, Inc. (TDC Americas) may be referred to in this protocol as "sponsor" or "Takeda".
Study Number:	TAK-676-1003
EudraCT Number:	Not applicable
Compound:	TAK-676
Date:	18 February 2022 **Amendment Number:** 2

Amendment History:

Date	Amendment Number	Region
18 February 2022	Amendment 2	North America
31 March 2021	Amendment 1	North America
23 October 2020	Initial protocol	North America

1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

A separate contact information list will be provided to each site.

Serious adverse event (SAE) and pregnancy reporting information is presented in Section 10.0, as is information on reporting product complaints.

Takeda Development Center–sponsored investigators per individual country requirements will be provided with emergency medical contact information cards to be carried by each patient.

General advice on protocol procedures should be obtained through the monitor assigned to the study site. Information on service providers is given in Section 3.1 and relevant guidelines provided to the site.

The names and contact information for the medical monitor and responsible medical officer are in the Study Manual.

1.2 Approval

REPRESENTATIVES OF TAKEDA

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Council for Harmonisation (ICH) E6 Good Clinical Practice (GCP): Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws, clinical trial disclosure laws, and regulations.

SIGNATURES

The signature of the responsible Takeda medical officer (and other signatories, as applicable) can be found on the signature page.

Electronic signatures may be found on the last page of this document.

[redacted], MD Date
[redacted],
Immuno-Oncology Research & Development
(or designee)

[redacted], PhD Date
[redacted],
SQS Oncology
(or designee)

[redacted], PhD Date
[redacted],
Quantitative Clinical Pharmacology
(or designee)

INVESTIGATOR AGREEMENT

I confirm that I have read and that I understand this protocol, the Investigator's Brochure (IB), prescribing information, and any other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also to protect the rights, safety, privacy, and well-being of study patients in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Council for Harmonisation, E6 Good Clinical Practice: Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Regulatory requirements for reporting serious adverse events defined in Section 10.0 of this protocol.
- Terms outlined in the clinical study site agreement.
- Responsibilities of the investigator (Appendix B).

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Appendix C of this protocol.

Signature of Investigator Date

Investigator Name (print or type)

Investigator's Title

Location of Facility (City, State/Province)

Location of Facility (Country)

1.3 Protocol Amendment 2 Summary of Changes

Protocol Amendment 2 Summary and Rationale:

This section describes the changes in reference to the protocol incorporating Amendment 2.

The primary reason for this amendment is to delineate the requirements and statistical considerations for paired biopsies.

In this amendment, minor grammatical, editorial, formatting, and administrative changes not affecting the conduct of the study are included for clarification and administrative purposes only.

Protocol Amendment 2			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
1.	Section 2.0 STUDY SUMMARY Section 6.1 Overview of Study Design Figure 6.a Study Design Section 6.1.1 Dose Escalation of TAK-676 Section 6.2 Number of Patients Section 6.3.1 Duration of an Individual Patient's Study Participation Section 13.3 Determination of Sample Size	Increased number of subjects to be enrolled and duration of the treatment.	Number of patients increased because of the addition of higher dose levels of TAK-676 to be investigated and increased duration of treatment to more closely align with pembrolizumab label.
2.	Section 4.5.1.2 TNBC	Updated details about IMPASSION 130 trial to justify selected patient population (triple-negative breast cancer [TNBC]). Also added information about the Keynote-355 trial, that it has been granted accelerated approval.	Updated section with current available information.
3.	Section 2.0 STUDY SUMMARY Section 4.5.3.1 Dose Range Figure 6.a Study Design Section 6.1 Overview of Study Design Section 8.1.3 TAK-676 Section 8.3 Dose Escalation Rules Table 8.d Planned Dose Levels of TAK-676 Section 13.3 Determination of Sample Size	Increased the explorable dose range for TAK-676, provided justification of increase in dose range, and explained dose escalation criteria in respective sections.	Increased the dose range on the basis of the preliminary safety/tolerability data available from preplanned interim safety data review in the first-in-human (FIH) Study TAK-676-1002 and allowed for more aggressive dose escalation on the basis of this data.

Protocol Amendment 2			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
4.	Section 2.0 STUDY SUMMARY Section 5.1.2 Secondary Objectives Section 5.2.2 Secondary Endpoints Section 5.1.3 Exploratory Endpoints Table 6.b Primary and Secondary Endpoints for Disclosures Section 13.1.5 Pharmacodynamic Analysis Appendix K Statistical Considerations for Paired Biopsies	Added secondary objective and respective endpoint for evaluation of dose-responsive impact on tumor immune cell infiltration and activation in nonirradiated tumors after TAK-676 administration.	To more closely align with American Society of Clinical Oncology (ASCO)'s Ethical Framework for Including Research Biopsies in Oncology Clinical Trials by providing clarity and quantification around mandatory biopsy collection and elevating it from exploratory to secondary objective/endpoint.
5.	Section 4.5.4.1 Tumor Biopsies Section 9.4.15.2.2 Fresh Paired Tumor Biopsy Appendix K Statistical Considerations for Paired Biopsies	Addition of statement for mandatory biopsies in all patients with safely accessible lesions, on observing pharmacodynamic activity. Addition of appendix to explain statistical considerations for paired biopsies.	To more closely align with ASCO's Ethical Framework for Including Research Biopsies in Oncology Clinical Trials by providing clarity and quantification around mandatory biopsy collection and elevating it from exploratory to secondary objective/endpoint.
6.	Appendix A Schedule of Events Table 1 and Table 2	Addition of blood sample collection at 6 hours after the end of infusion of TAK-676 in Cycle 1 Day 1 (C1D1) and C1D15 for immunophenotyping.	On-treatment blood samples are collected to look at activation of peripheral immune cells.
7.	Section 2.0 STUDY SUMMARY Section 5.2.2 Secondary Endpoints	Revised response assessments to include evaluation using both Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 and modified intratumoral immunotherapy Response Evaluation Criteria in Solid Tumors (itRECIST).	Clarification.

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8.	Section 2.0 STUDY SUMMARY Section 5.2.2 Secondary Endpoints Section 9.4.14 Disease Assessment Section 9.4.14.1.1 Irradiated and Nonirradiated Response Figure 9.a Illustration of Irradiated, Nonirradiated, and Overall Responses per Modified itRECIST	Addition of use of itRECIST criteria for select response assessments. Per itRECIST: overall response rate (confirmed complete response + confirmed partial response), duration of response, time to response.	Clarify the need to assess response both per RECIST v.1.1 as well as per modified itRECIST criteria to allow for assessment of irradiated and nonirradiated lesions.
9.	Section 6.1 Overview of Study Design Section 8.1.1 Radiation Treatment Regimen Section 9.4.14 Disease Assessment	Added a clarifying statement that before radiation therapy, the radiology oncologist and medical oncologist should agree on the target lesions identified for irradiation using the baseline imaging assessments.	Addition made to provide more clarity.
10.	Section 2.0 STUDY SUMMARY Section 7.1 Inclusion Criteria Section 9.4.14.1.1 Irradiated and Nonirradiated Response	Modified Inclusion Criteria #4 to add dimensions of measurable tumor lesions.	Addition made to provide more clarity around what is considered a measurable lesion.
11.	Section 2.0 STUDY SUMMARY Section 7.1 Inclusion Criteria	Inclusion criteria #6 on life expectancy of the patient made not applicable.	While investigators are still expected to use their best judgment in enrolling patients who they expect could remain on study reasonably long enough to assess safety and efficacy of study treatments, it was determined that being able to quantitate life expectancy is quite difficult, thus it was removed as a specific criterion for inclusion.
12.	Section 2.0 STUDY SUMMARY Section 7.2 Exclusion Criteria Section 8.5 Excluded Concomitant Medications and Procedures	The 14-day period of time off systemic steroids before C1D1 start was reduced to 7 days.	Because these steroids have a short half-life, a shorter wash-out period is better justified and optimal.

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13.	Section 8.1.1 Radiation Treatment Regimen Appendix A Schedule of Events Table 1	Revised the time interval for radiation therapy administration to between Day -8 to Day -2 instead of between Day -8 to Day -1	Revision made to ensure minimum 40 hours of time interval between end of radiation therapy and initiation of pembrolizumab infusion.
14.	Section 8.1.3 TAK-676 Section 8.8.2 CRS	Highlighted the option to use other anti–IL-6 agents for cytokine release syndrome (CRS) treatment due to limited availability of tocilizumab.	Due to the limited supply of tocilizumab, which has been publicly stated by the manufacturer, an alternative for tocilizumab in treatment of CRS is noted.
15.	Section 8.1.3 TAK-676 Appendix A Schedule of Events Table 1 footnotes	Clarified that TAK-676 infusion will start 1 hour (+15 minutes) after the end of pembrolizumab infusion.	Revised language to bring more clarity.
16.	Section 8.8.12 Management of COVID-19–Positive Patients	In the text about symptomatic patients who test positive for coronavirus disease 2019 (COVID-19) during screening or enrollment, the language was updated to require resolution of symptoms and completion of any COVID-19 treatments within 7 days of enrollment or retreatment with study drugs. In the text about asymptomatic patients who test positive for COVID-19 during screening or enrollment, the language was updated to require a 7-day waiting period from the time of positive test before enrollment or retreatment. In addition, a negative COVID-19 polymerase chain reaction test is no longer a requirement for enrollment or retreatment in symptomatic or asymptomatic patients unless required per local guidelines. Also included consultation with medical monitor.	In response to evolving COVID-19 testing recommendations and treatment options.

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17.	Table 8.f Guidelines for TAK-676 Dose Modification and/or Discontinuation for Nonhematologic Toxicity	Updated dose modification for hepatoxicity.	Update made to ensure consistency within the protocol.
18.	Section 8.6 Permitted Concomitant Medications and Procedures	Added oxygen supplementation.	To ensure it gets recorded as a concomitant medication.
19.	Section 8.8.1 Pulmonary Vasculature Toxicity/Noncardiogenic Pulmonary Edema Table 8.h Pulmonary Toxicity Clinical Management Recommendations	Revised dose modification suggestion in the setting of pulmonary toxicity.	Update made to ensure consistency within the protocol and to not unnecessarily hold study drug based on an isolated oxygen saturation <95%.
20.	Table 8.iNCCN Guidelines Version 3.2021: Management of CAR T Cell–Related Toxicities	Modified table based on most current NCCN guidelines for management of chimeric antigen receptor T cell–related toxicities.	Update made to use more current guidance.
21.	Section 8.8.2 CRS	The dose modification due to CRS was clarified to indicate that if TAK-676 is discontinued, pembrolizumab should be held until the CRS event recovers to Grade ≤1.	Clarification.
22.	Section 8.8.6 Nausea and/or Vomiting Section 8.8.7 Diarrhea	Addition of statement to use evolving consensus guidelines for management of immune mediated toxicity, if nausea/vomiting/diarrhea are thought to be immune mediated.	Additional references added for management of clinical events.
23.	Section 8.8.10 Management of Pembrolizumab Immune-Mediated AEs	Addition of statement to use evolving consensus guidelines for management of immune-checkpoint inhibitor–related adverse events, in case of immune-mediated reactions due to pembrolizumab administration.	Additional references added for management of clinical events.
24.	Section 9.3 Treatment Group Assignments	Changed the following sentence to past tense as current study is now ongoing: Safety data was to be obtained from at least 2 dose cohorts of the FIH combination arm before administering TAK-676 with pembrolizumab following radiation therapy in the current study.	Editorial update.

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25.	Table 9.b Clinical Urinalysis Tests	Leukocytes was corrected to leukocyte esterase.	Correction.
26.	Section 9.4.14 Disease Assessment	The scan of the neck was clarified to be done only for patients with head and neck squamous cell carcinoma and when clinically indicated for patients with TNBC and non–small-cell lung cancer. The anatomy required at follow-up time points was also clarified.	Clarification.
27.	Section 9.4.14.1.2 Treatment Beyond Progression	Clarified that treatment beyond progression will be based on RECIST v.1.1 criteria and not modified itRECIST.	Clarification.
28.	Section 9.4.15.3 Biomarker and Pharmacodynamic Measurements	Included expression analysis for blood sample RNA and inclusion of statements that measurements will comply with local regulations.	Clarification.
29.	Section 9.10 Study Compliance	Updated window between radiation treatment and start of planned study drug(s) administration, in case if AE occurs after radiation treatment.	This specified duration of delay in treatment start defines an upper limit of reasonable delay, beyond which the potential implications for patient safety and study validity necessitate discussion with the Medical Monitor.
30.	Section 13.1.1 Analysis Sets	Addition of comprehensive response-evaluable analysis set as subset of safety analysis set and conversion of response evaluable analysis set as subset of safety and comprehensive response-evaluable analysis set.	Change made to to have an additional way of defining the denominator when calculating the response rates.
31.	Section 13.1.7 ECG Analysis	Assessment of QT interval with Bazett correction method was removed.	The assessment of QT interval by Fridericia formula is enough.

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32.	Appendix A Schedule of Events Table 1	Modified footnotes d and added footnote e to clarify that vital signs and Eastern Cooperative Oncology Group performance status should also be performed before the first fraction of radiation therapy.	Clarification.
33.	Appendix E BOIN Design	Added instructions for when additional dose levels are added.	To align with other protocol changes that allow for investigation of higher dose levels.
34.	Appendix J Dose constraints as per Task Group-101	Fixed table misalignment.	Correction.

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2.0 STUDY SUMMARY

Name of Sponsor(s): Takeda Development Center Americas, Inc.	**Compound:** TAK-676
Title of Protocol: An Open-Label, Phase 1, Dose-Escalation Study to Evaluate the Safety and Preliminary Antitumor Activity of TAK-676 With Pembrolizumab Following Radiation Therapy in the Treatment of Non–Small-Cell Lung Cancer, Triple-Negative Breast Cancer, or Squamous-Cell Carcinoma of the Head and Neck that Has Progressed on Checkpoint Inhibitors.	**EudraCT No.**: Not applicable
Study Number: TAK-676-1003	**Phase:** 1
Study Design: This is an open-label, phase 1, dose escalation study to evaluate the safety, tolerability, and preliminary antitumor activity of TAK-676 and pembrolizumab following radiation therapy in the treatment of non–small-cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), or squamous-cell carcinoma of the head and neck (SCCHN) patients who have progressed on checkpoint inhibitors (CPIs). Approximately 65 patients with metastatic NSCLC, TNBC, or SCCHN will be enrolled in this study, to achieve a maximum of 55 dose-limiting toxicity (DLT) evaluable patients. All patients will receive 8 Gy × 3 doses of image-guided radiation therapy, followed by intravenous (IV) administration of pembrolizumab and TAK-676. Pembrolizumab will be administered at 200 mg IV on Day 1 of every 21-day cycle, with a minimum for 40 hours between the last fraction of radiation therapy and the initiation of IV pembrolizumab. TAK-676 will be administered in a dose-escalating fashion following the Bayesian Optimal INterval (BOIN) design, with an explorable dose range of 0.2 to 9.0 mg administered on Days 1, 8, and 15 of every 21-day cycle. Patients will only receive TAK-676 with pembrolizumab at dose levels that were previously deemed safe in the dose-finding phase 1 study TAK-676-1002. Three patients will be enrolled in the initial cohort at the previously identified starting dose level of TAK-676. Subsequent cohorts will enroll 2 to 3 patients per escalation/de-escalation guidelines. Administration of pembrolizumab (every 3 weeks) and TAK-676 (weekly) will continue until disease progression, intolerance to pembrolizumab or TAK-676 (defined as the development of a treatment-emergent adverse event [TEAE] that is at least possibly related to pembrolizumab or TAK-676 and for which dose discontinuation is recommended), or withdrawal of consent, whichever occurs first.	
Primary Objective: The primary objective is: • To determine the safety and tolerability of TAK-676 administered in combination with pembrolizumab following radiation therapy in patients with locally advanced or metastatic NSCLC, TNBC or SCCHN.	
Secondary Objectives: The secondary objectives are: • To determine the recommended phase 2 dose (RP2D) of TAK-676 administered in combination with pembrolizumab following radiation therapy. RP2D can be equal to or lower than the maximum tolerated dose (MTD). • To assess the preliminary antitumor activity of TAK-676 administered in combination with pembrolizumab following radiation therapy, both locally (in the radiation field) and systemically (nonirradiated lesions). • To evaluate the dose-responsive impact on T-cell infiltration in nonirradiated tumors following TAK-676 administered in combination with pembrolizumab following radiation therapy.	
Patient Population: Healthy male or female patients aged 18 years or older, with advanced NSCLC, TNBC, or SCCHN, who have progressed on CPIs.	
Number of Patients: Approximately 65 patients will be enrolled in this study to achieve a	**Number of Sites:** Estimated total: Approximately 6

maximum of 55 DLT evaluable patients.	sites in North America.
Dose Level(s): Radiation therapy: Fractionated dose of 8 Gy × 3. Pembrolizumab: 200 mg. TAK-676: 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.5, 3.5, 5.0, 7.0, and 9.0 mg.	**Route of Administration:** Pembrolizumab: IV infusion over 30 minutes. TAK-676: IV infusion over 60±10 minutes.
Duration of Treatment: Approximately 24 months. Patients with demonstrated clinical benefit will continue treatment beyond this point if approved by the sponsor.	**Period of Evaluation:** It is anticipated that this study will last for approximately 30 months.

Inclusion Criteria:

1. Adult male or female patients, aged 18 years or older.
2. Voluntary written consent must be given before performance of any study-related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.
3. Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1.
4. Patients must have at least 2 measurable lesions (ie, ≥10 mm longest diameter for extranodal lesions, ≥15 mm short axis for lymph nodes) with at least one inside and at least one other outside of the radiation field. The tumor outside the radiation field must be accessible for biopsy, and the patient must consent to tumor biopsy at screening and during treatment as described in the SOE.
5. Patients with pathologically confirmed (cytological diagnosis is adequate) advanced or metastatic NSCLC, TNBC, or SCCHN who have:
   a) Received or been offered all established standard of care treatment options for which they are eligible; and
   b) Progressed on checkpoint inhibitors in a prior line of therapy.
6. Not Applicable. In Protocol Amendment 2, requirement of patient having life expectancy >12 weeks has been removed.
7. Adequate bone marrow, renal, and hepatic functions, as determined by the following laboratory parameters:
   a) Absolute neutrophil count (ANC) ≥1000/μL, platelet count ≥75,000/μL, and hemoglobin ≥8.0 g/dL without growth factor support for neutrophils or transfusion support for platelets within 14 days before the first study treatment dose.
   b) Total bilirubin ≤1.5 times the institutional ULN. For patients with Gilbert's disease, ≤3 mg/dL.
   c) Serum ALT and AST ≤3.0 times the ULN (or ≤5.0 times the ULN with presence of liver metastases).
   d) Albumin ≥3.0 g/dL.
   e) Estimated creatinine clearance using the Cockcroft-Gault formula ≥30 mL/min.
8. Left ventricular ejection fraction (LVEF) >50%, as measured by echocardiogram or multiple-gated acquisition (MUGA) scan within 4 weeks before receiving the first dose of study drug.
9. Clinically significant toxic effects of previous therapy have recovered to Grade 1 (per NCI CTCAE, Version 5.0) or baseline, except for alopecia, Grade 2 peripheral neuropathy, and/or autoimmune endocrinopathies with stable endocrine replacement therapy.
10. Female patients must be:
    a) Postmenopausal (natural amenorrhea and not due to other medical reasons) for at least 1 year before the screening visit, OR
    b) Surgically sterile, OR
    c) If they are of childbearing potential, agreeable to practicing 2 effective methods of contraception at the same time, from the time of signing the informed consent through 120 days after the last dose of study drug(s), OR
    d) Agreeable to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient.

    Note: Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods), withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.
11. Male patients, even if surgically sterilized (ie, status postvasectomy), must:

a) Agree to practice effective barrier contraception during the entire study treatment period and through 120 days after the last dose of study drug, OR
b) Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient.

Note: Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods), withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.

Exclusion Criteria:

1. History of any of the following ≤6 months before first dose of study drug(s): congestive heart failure New York Heart Association Grade III or IV, unstable angina, myocardial infarction, persistent hypertension ≥160/100 mm Hg despite optimal medical therapy, ongoing cardiac arrhythmias of Grade >2 (including atrial flutter/fibrillation or intermittent ventricular tachycardia), other ongoing serious cardiac conditions (eg, Grade 3 pericardial effusion or Grade 3 restrictive cardiomyopathy), or symptomatic cerebrovascular events. Chronic, stable atrial fibrillation on stable anticoagulation therapy, including low molecular-weight heparin, is allowed.
2. History of brain metastasis unless:
   a) Clinically stable, (ie, treatment completed ≥4 weeks prior) following prior surgery, whole-brain radiation, or stereotactic radiosurgery, AND
   b) Off corticosteroids.
3. Known history of uncontrolled autoimmune disorders, HIV infection, or other relevant congenital or acquired immunodeficiencies.
4. Chronic, active hepatitis (eg, patients with known hepatitis B surface antigen seropositive and/or detectable hepatitis C virus [HCV]-RNA).

   Note: Patients who have positive hepatitis B core antibody can be enrolled but must have an undetectable serum hepatitis B virus-DNA. Patients who have positive HCV antibody must have an undetectable HCV-RNA serum level.
5. Contraindication and/or history of intolerance to the administration of CPI.
6. Contraindication and/or history of intolerance to the radiation therapy.
7. Any illness, metabolic dysfunction, physical examination finding, or clinical laboratory finding that give reasonable suspicion of a disease or condition that would contraindicate the use of an investigational drug or that would limit compliance with study requirements or compromise ability to provide written informed consent.
8. Treatment with any investigational products and systemic anticancer drugs (including vascular endothelial growth factor inhibitors), within 14 days or 5 half-lives, whichever is shorter, before Cycle 1 Day 1 (C1D1) of study drug(s).
9. Concurrent chemotherapy, immunotherapy (except for pembrolizumab), biologic, or hormonal therapy (except for adjuvant endocrine therapy for a history of breast cancer). Concurrent use of hormones for noncancer-related conditions is acceptable (eg, corticosteroid replacement use).
10. Prior radiation to lesions chosen for biopsy or response assessment.
11. Prior radiation to lesions other than those chosen for radiation therapy or biopsy in the current protocol within 4 weeks of C1D1 of study drug(s).
12. Use of systemic corticosteroids or other immunosuppressive therapy, concurrently or within 7 days of start of radiation therapy, with the following exceptions:
    a) Topical, intranasal, inhaled, ocular, intra-articular and/or other nonsystemic corticosteroids.
    b) Physiological doses of replacement steroid therapy (eg, for adrenal insufficiency).
13. Receipt of live attenuated vaccine (eg, tuberculosis Bacillus Calmette-Guerin [BCG] vaccine, oral polio vaccine, measles, rotavirus, yellow fever) within 28 days of C1D1 of study drug(s).
14. Recipients of allogeneic or autologous stem cell transplantation or organ transplantation.
15. Female patients who are lactating or have a positive serum pregnancy test during the screening period or a positive urine pregnancy test on Day 1 before first dose of study drug.

    Note: Female patients who are lactating will be eligible if they choose to discontinue breastfeeding before the first dose of study drug.
16. Ongoing Grade ≥2 infection or patients with Grade ≥2 fever of malignant origin.

17. Fridericia's corrected QT interval (QTcF) >450 milliseconds (msec) (males) or >475 msec (females) on a 12-lead ECG during the screening period.
18. Grade ≥2 hypotension (ie, hypotension for which nonurgent intervention is required) at screening or during C1D1 predose assessment.
19. Oxygen saturation <92% on room air at screening or during C1D1 predose assessment.
20. Use of medications that are known clinical OATP1B1 and/or OATP1B3 inhibitors, concurrently or within 14 days of C1D1 of study drug(s).
21. Patients treated with other STING agonists/antagonist and toll-like receptors agonists within the past 6 months.
22. Current smoker.
23. Vaping within 90 days of C1D1 of study drug(s).
24. Current diagnosis of pneumonitis, interstitial lung disease, severe chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, other restrictive lung diseases, acute pulmonary embolism, or Grade ≥2 pleural effusion or ascites not controlled by tap or requiring indwelling catheters.

Main Criteria for Evaluation and Analyses:

The primary endpoints are:

- Frequency and severity of treatment-emergent adverse events (TEAEs).
- Number of patients with DLTs.
- Number/percentage of patients with 1 or more treatment-emergent serious adverse event (TESAE).
- Number/percentage of patients with 1 or more TEAE leading to dose modifications and treatment discontinuation.

Safety endpoints will be evaluated according to the NCI CTCAE, Version 5.0.

The secondary endpoints are:

Response assessments are per Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 by investigator.

- Overall response rate (ORR): confirmed complete response [cCR] + confirmed partial response [cPR].
- Duration of response (DOR) for all tumor lesions assessed by RECIST v.1.1.
- Time to response (TTR) for all tumor lesions assessed by RECIST v.1.1

Response assessments are per modified intratumoral immunotherapy Response Evaluation Criteria in Solid Tumors by investigator.

- Overall response rate (ORR): cCR + cPR.
- Overall response rate for tumors lying within the radiation field (ORRirradiated): confirmed complete response [cCRirradiated] + confirmed partial response [cPRirradiated] of tumor lesions lying within the radiation field.
- Overall response rate for tumors lying outside the radiation field (ORRnonirradiated): confirmed complete response [cCRnonirradiated] + confirmed partial response [cPRnonirradiated] of tumor lesions lying outside of the radiation field.
- DOR for tumors lying within the radiation field (DORirradiated), and for those lying outside of the radiation field (DORnonirradiated).
- TTR for tumors lying within the radiation field (TTRirradiated), and for those lying outside of the radiation field (TTRnonirradiated).
- The following endpoint will be assessed to evaluate T-cell infiltration into the tumor between pretreatment and on-treatment biopsies:
  - Cell infiltration evaluated by immunohistochemistry.

Statistical Considerations:

Dose escalation of TAK-676 will follow BOIN design to inform dose escalation decision and potential MTD estimation. Three patients will be enrolled in the initial cohort at the previously identified starting dose level of

TAK-676. Subsequent cohorts will enroll 2 to 3 patients per the escalation/de-escalation guidelines. The target toxicity rate is ϕ= 0.3. It is generally expected that at least 3 patients will enroll per cohort. However, if no DLTs have been identified in the TAK-676 + pembrolizumab dose level that has already been evaluated in the dose-finding phase 1 TAK-676-1002 study, the sponsor, in agreement with the TAK-676-1003 investigators, may opt to enroll a 2-patient cohort at that same [radiation] + TAK-676 + pembrolizumab dose level being evaluated in the TAK-676-1003 study. To guide dose escalation decisions, if the observed DLT rate at the current dose is ≤0.236, the next cohort of patients will be treated at the next higher dose level; if it is ≥0.358, the next cohort of patients will be treated at the next lower dose level; if it is within 0.236 and 0.358, additional patients will be enrolled in this dose level. For the purpose of overdose control, dose j and higher levels will be eliminated from further examination if $Pr(p_j>0.3 \mid data) >0.95$, where p_j is the true DLT rate of dose level j. When the lowest dose is eliminated, the dose escalation will be stopped for safety. Dose escalation will continue until the maximum sample size (55 DLT-evaluable patients) is reached or the number of DLT evaluable patients treated at the current dose reaches 9. Isotonic regression method will be used on the cumulative DLT rate for each dose level to determine the MTD, defined as the highest TAK-676 dose in combination with radiation therapy and pembrolizumab that does not result in unacceptable toxicities.

Dose Escalation/De-escalation Rule for the BOIN Design

Number of patients treated at the current dose	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Escalate if number of DLT ≤	0	0	0	0	1	1	1	1	2
Deescalate if number of DLT ≥	1	1	2	2	2	3	3	3	4
Eliminate if number of DLT ≥	NA	NA	3	3	4	4	5	5	5

Number of DLT is the number of patients with at least 1 DLT.

Other dose escalation decisions, evaluation of intermediate doses, expansion of an existing dose level, and stopping the dose escalation early are all permissible following discussions between the sponsor and the investigators, if such measures are needed for patient safety, or for a better understanding of the dose-related toxicity, exposure, and/or pharmacodynamics. Other non-DLT safety and available clinical, pharmacokinetic, or biomarker data will also be considered to inform subsequent dose recommendations, dose escalation decisions, and potential MTD and RP2D estimation.

Safety will be evaluated by the frequency of AEs, severity and types of AEs, AEs leading to dose modifications and treatment discontinuations, and other safety endpoints using the safety analysis set. The incidence of DLTs will be tabulated using the DLT-evaluable analysis set. ORR, ORRirradiated, and ORRnonirradiated will be summarized using descriptive statistics with 95% CI for the response-evaluable analysis set. DOR, DORirradiated, DORnonirradiated, TTR, TTRirradiated, and TTRnonirradiated will be analyzed descriptively using Kaplan-Meier method for response-evaluable analysis set.

Sample Size Justification: Approximately 65 patients with metastatic NSCLC, TNBC, or SCCHN will be enrolled in this study, to achieve a maximum of 55 DLT evaluable patients. They will receive pembrolizumab administered at 200 mg IV on Day 1 of every 21-day cycle, and TAK-676 administered in a dose-escalating fashion following the BOIN design, with an explorable dose range of 0.2 to 9.0 mg administered on Days 1, 8, and 15 of every 21-day cycle.

3.0 STUDY REFERENCE INFORMATION

3.1 Study-Related Responsibilities

The sponsor will perform all study-related activities with the exception of those identified in the clinical supplier list in the Study Manual. The identified vendors will perform specific study-related activities either in full or in partnership with the sponsor.

3.2 Principal Investigator/Coordinating Investigator

Takeda will select a signatory coordinating investigator from the investigators who participate in the study. Selection criteria for this investigator will include significant knowledge of the study protocol, the study medication, their expertise in the therapeutic area and the conduct of clinical research, and study participation. The signatory coordinating investigator will be required to review and sign the clinical study report (CSR) and by doing so agrees that it accurately describes the results of the study.

3.3 List of Abbreviations

AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
anti-PD-1	anti-programmed cell death protein 1
anti-PD-L1	anti-programmed cell death ligand 1
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC_{24}	area under the concentration-time curve from time 0 to 24 hours
BIW	twice weekly
BOIN	Bayesian Optimal INterval
BWL	body weight loss
cCR	confirmed complete response
cCRirradiated	confirmed complete response in tumors lying within the radiation field
cCRnonirradiated	confirmed complete response in tumors lying outside the radiation field
CDN	cyclic dinucleotide
CFR	Code of Federal Regulations
cGAMP	cyclic guanosine monophosphate–adenosine monophosphate or cyclic GMP-AMP
cGAS	cyclic GMP-AMP synthase
C_{max}	maximum observed concentration
COVID-19	coronavirus disease 2019
CPI	checkpoint inhibitor
CPK	creatine phosphokinase
cPR	confirmed partial response
cPRirradiated	confirmed partial response in tumors lying within the radiation field
cPRnonirradiated	confirmed partial response in tumors lying outside the radiation field
CRO	contract research organization
CRS	cytokine release syndrome
CSR	clinical study report
CT	computed tomography
CTV	clinical tumor volume
CxDx	Cycle x Day x (eg, C1D1: Cycle 1 Day 2)
CYP	cytochrome P-450
DC	dendritic cell
DDI	drug-drug interaction
DLT	dose-limiting toxicity
DOR	duration of response
DORirradiated	duration of response in tumors lying within the radiation field

DORnonirradiated	duration of response in tumors lying outside the radiation field
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture
EOS	end of study
EOT	end of treatment
FDA	[United States] Food and Drug Administration
FIH	first-in-human
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor
GI	gastrointestinal
GIT	gastrointestinal tract
GLP	Good Laboratory Practice
GTV	gross tumor volume
Gy	gray
HCV	hepatitis C virus
hERG	human ether-à-go-go–related gene
HNSTD	highest nonseverely toxic dose
IB	Investigator's Brochure
ICB	immune checkpoint blockade
ICF	informed consent form
ICH	International Council for Harmonisation
ICU	intensive care unit
IEC	independent ethics committee
IFN	interferon
IL	interleukin
IP-10	interferon gamma inducible protein
IRB	institutional review board
IRF3	interferon regulatory factor 3
itRECIST	intratumoral immunotherapy Response Evaluation Criteria in Solid Tumors
ITV	internal target volume
IV	intravenous(ly)
LVEF	left ventricular ejection fraction
MABEL	minimum anticipated biological effect level
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	[United Kingdom] Medicines and Healthcare products Regulatory Agency
MRI	magnetic resonance imaging
mRNA	messenger RNA
MRSD	maximum recommended starting dose
msec	milliseconds

MTD	maximum tolerated dose
MUGA	multiple-gated acquisition
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK	natural killer
NOAEL	no-observed-adverse-effect level
NSCLC	non–small-cell lung cancer
NT-I	nontarget irradiated
NT-NI	nontarget nonirradiated
OAR	organs at risk
OATP	organic anion-transporting polypeptide
ORR	overall response rate
ORRirradiated	overall response rate for tumors lying within the radiation field
ORRnonirradiated	overall response rate for tumors lying outside the radiation field
PCR	polymerase chain reaction
PD	progressive disease
PD-1	programmed cell death protein 1
PD-L1	programmed cell death ligand 1
PFS	progression-free survival
PK	pharmacokinetic(s)
PMDA	Pharmaceuticals and Medical Devices Agency, Japan
PR	partial response
PTV	planning tumor volume
Q3D	once every 3 days
QTcF	QT interval with Fridericia correction method
QW	once per week
RBC	red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	recommended phase 2 dose
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus-2
SBRT	stereotactic body radiation therapy
SCCHN	squamous cell carcinoma of head and neck
SD	stable disease
SOC	standard of care
SOE	schedule of events
SRS	stereotactic radiosurgery
STING	Stimulator of Interferon Genes
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
T-I	target irradiated

TME	tumor microenvironment
TNBC	triple negative breast cancer
[redacted]	[redacted]
T-NI	target, nonirradiated
TTR	time to response
TTRirradiated	time to response in tumors lying within the radiation field
TTRnonirradiated	time to response in tumors lying outside the radiation field
UK	United Kingdom
ULN	upper limit of normal
US	United States
V_{ss}	volume of distribution at steady state after intravenous administration
WHO	World Health Organization

3.4 Corporate Identification

Millennium	Millennium Pharmaceuticals, Inc, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited
TDC Japan	Takeda Development Center Japan
TDC Asia	Takeda Development Center Asia, Pte Ltd
TDC Europe	Takeda Development Centre Europe Ltd
TDC Americas	Takeda Development Center Americas, Inc
TDC	TDC Japan, TDC Asia, TDC Europe and/or TDC Americas, as applicable
Takeda	Millennium Pharmaceuticals, Inc, TDC Japan, TDC Asia, TDC Europe and/or TDC Americas, as applicable

4.0 INTRODUCTION

Overcoming T cell inhibition in the tumor microenvironment (TME) with checkpoint inhibitors (CPIs) has proven to be a successful strategy to produce long-term benefit in a significant number of patients with metastatic solid tumors. Despite these advances, many patients with advanced cancer are either refractory to CPIs or relapse after a period of tumor control, eventually succumbing to their disease. Evolving data suggest that reduced interferon (IFN) signaling, immune escape through human leukocyte antigen loss, as well as altered antigen presentation may contribute to CPI resistance (Jenkins et al. 2018; Minn and Wherry 2016; Sharma et al. 2017). Furthermore, an emerging consensus acknowledges that CPI resistance (relapse or refractory in nature) may also be driven by tumor immunophenotype, specifically those tumors harboring an immunosuppressive or "immune desert" phenotype. Accordingly, one possible strategy to overcome these elements of resistance is to stimulate innate immune cells (myeloid cells, including antigen-presenting dendritic cells [DCs], granulocytes, eosinophils, neutrophils, or monocytes/macrophages, as well as lymphoid cells, including gamma/delta T cells, natural killer [NK] cells, and NK T Cells) to condition the TME, thus turning immunologically "cold" tumors into "hot" tumors in which adaptive immune responses can be effectively activated.

Study TAK-676-1003 intends to evaluate the safety, tolerability, and preliminary efficacy of radiation therapy followed by the combination of pembrolizumab and the Stimulator of Interferon Genes (STING) agonist TAK-676 as a means to stimulate an immune-mediated antitumor response in otherwise immunologically cold tumors.

4.1 STING Signaling and Innate and Adaptive Immune Response

STING is a cytosolic protein critical for the induction of type I IFN-dependent innate immunity (Ishikawa et al. 2009) and is specifically activated in the presence of cyclic dinucleotides (CDNs) derived from bacteria or produced by cyclic GMP-AMP synthase (cGAS) (Sun et al. 2013; Wu et al. 2018). Emerging evidence indicates that the cGAS-STING pathway plays an important role specifically in driving tumor surveillance (Corrales and Gajewski 2015). The presence of cytosolic DNA in tumor cells leads to cGAS-dependent production of the second messenger 2′,3′-cyclic guanosine monophosphate–adenosine monophosphate (cGAMP), which may subsequently be transmitted to professional antigen-presenting cells in the TME. Ultimately, this leads to induction of type I IFNs in DCs (Marcus et al. 2018). Type I IFNs and downstream regulated cytokines/chemokines serve as a potent "alert" to immune cells, including T cells and NK cells that may prime antitumor responses. Downregulation of cGAS via epigenetic silencing has been reported in some human tumors, underscoring the potential significance of STING signaling in immune evasion (Konno et al. 2018).

4.2 Radiation Therapy and the Innate and Adaptive Immune Response

Radiotherapy is a pillar of cancer treatment with approximately 50% of cancer patients receiving radiotherapy at some point in the course of their disease (Delaney et al. 2005). In the setting of metastatic disease of solid tumors, including non–small-cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), and squamous cell carcinoma of head and neck (SCCHN), radiotherapy is

often used with palliative intent. Technological advances have allowed radiotherapy to be delivered more precisely with such techniques as 3D conformal radiotherapy, image-guided intensity-modulated radiotherapy, and stereotactic ablative radiotherapy (Ahmad et al. 2012). With the increasing use of CPIs in these patients and the available evidence that radiation may enhance the immune response through activation of the STING pathway, there is much enthusiasm about combining radiation and CPI with the goal of improving treatment outcomes (Vatner et al. 2014). Radiotherapy has often been thought of as immunosuppressive due to the large treatment fields depleting peripheral lymphocytes through direct cell killing (Yovino et al. 2013). However, there is clinical evidence that focal radiation in conjunction with immune checkpoint blockade (ICB) can lead not only to reduced tumor size in the radiation field but also to a systemic disease response, termed the "abscopal response," in patients with metastatic disease, including NSCLC (Formenti et al. 2018; Vatner et al. 2014). This is thought to be through the release of tumor antigens by radiation-induced cell death in the context of endogenous adjuvants that facilitate priming of antitumor lymphocytes (Golden et al. 2012). This mechanism of radiation-induced immunogenicity involves the facilitation of tumor antigen uptake by DC and cross-presentation on major histocompatibility complex I, which is shown to be upregulated by radiotherapy in preclinical models (Formenti and Demaria 2013; Reits et al. 2006). This enhanced presentation of antigens released by cytotoxic radiotherapy potentiates cross-priming of tumor-specific lymphocytes in the lymph nodes, which may potentially prime an exhausted immune system in patients who are progressing on immunotherapy.

Local radiotherapy has been demonstrated to stimulate antitumor immune responses by increasing both apoptosis of tumor cells and subsequent antigen presentation and corresponding expression of immunomodulatory genes (Liao et al. 2004). Local radiation may also work through a different mechanism, that of altering the TME to promote greater infiltration by immune effector cells (Lugade et al. 2008; Matsumura et al. 2008; Zhang et al. 2007). Several studies have observed that the rapid reduction of tumor burden after ablative radiation therapy depends on T cell responses (Gupta et al. 2012; Lee et al. 2009). Ablative radiation also increases T cell priming in draining lymphoid tissues and reduces both the primary tumor and distant metastasis in a CD8 T cell–dependent fashion (Figure 4.a).

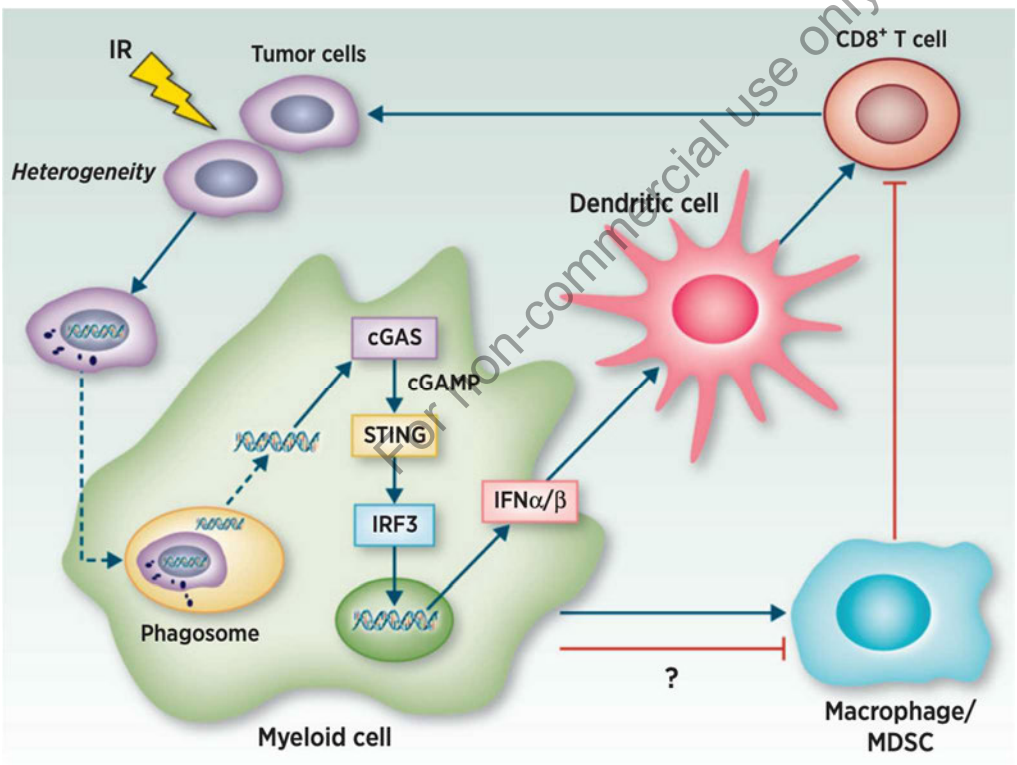
Preclinical tumor models have demonstrated that the immunogenic consequences of radiation are dependent on the cGAS/STING pathway (Deng et al. 2014b). Radiation-induced DNA damage leads to the generation of cytosolic DNA, which is sensed by cGAS. cGAS then catalyzes the production of cGAMP, which activates STING in tumor cells and nearby DCs, resulting in downstream induction of IFN-β (Deng et al. 2014b; Vanpouille-Box et al. 2017). IFN-β leads to enhanced antigen uptake by DCs and cross-presentation to T cells (Deng et al. 2014b). This recently redefined mechanism has bridged the tumor DNA damage response and host cell cytosolic DNA-sensing pathways in the context of radiotherapy (Figure 4.a).

The efficacy of radiotherapy has been shown to depend on the host immune response, particularly the adaptive immune response. By inducing apoptosis/necrosis of tumor tissues and subsequent release of DNA fragments and other danger signals, local high-dose radiation promotes inflammation (Apetoh et al. 2007). The potential role of type I IFN in bridging the inflammation induced by radiation has been investigated, demonstrating that type I IFNs result in a

tumor-reducing adaptive immune response (Burnette et al. 2011) (Figure 4.a). It has also been shown that radiation increases the production of IFNβ in the TME. The implications of this increase are significant. The therapeutic effect of radiation is diminished in an IFNα/β receptor knockout host (Burnette et al. 2011).

Preclinical work in tumor-bearing mice treated with radiation and intratumoral STING agonists injection provides evidence for an immune-mediated combination benefit. Deng et al. demonstrated that intratumoral delivery of the STING agonist 2′3′-cGAMP potentiates the response of MC38 tumors to radiation, and this combination benefit is dependent on the presence of STING in the host mice (Deng et al. 2014b). Similarly, a synthetic STING agonist (RR-CDG) delivered intratumorally to Panc02 tumors potentiated the effects of radiation to these tumors, and the combination resulted in systemic T cell–mediated tumor suppression, including at distant tumor sites (Baird et al. 2016).

Figure 4.a Radiation and Local Induction of Type I Interferon



Source: Deng et al, 2016 (Deng et al. 2016).

Cytosolic DNA sensing orchestrates tumor immunity during radiotherapy. Radiation results in the upregulation of "find-me" and "eat-me" signals from tumor cells. During phagocytosis in myeloid cells, the DNA fragments hidden in irradiated tumor cells are released from phagosomes to cytoplasm, acting as a danger signal. The cyclase cGAS binds this DNA, becomes catalytically active, and generates cGAMP as a second messenger. cGAMP binds to STING, which in turn

activates IRF3 to induce type I IFN production. Type I IFN signaling in DCs promotes the crosspriming of CD8 T cells, leading to tumor control.

Because type I IFN is a potent inducer of programmed cell death ligand 1 (PD-L1), it was postulated that (i) radiation resistance could be due to the engagement of T cell–negative regulatory pathways, especially IFN-induced PD-L1 upregulation inside the tumor tissues; and (ii) anti-programmed cell death ligand 1 (anti-PD-L1) treatment could overcome resistance to radiation therapy, further helping in eradicating the tumor (Deng et al. 2014a). This has led to postulating that programmed cell death protein 1 (PD-1)/PD-L1 inhibitors may be effective in potentiating the effect of radiation therapy.

4.3 Radiation Therapy in Combination With CPI

Multiple trials have studied the combination of varying doses and schedules of radiation therapy with CPIs (primarily pembrolizumab, ipilimumab, and nivolumab) in metastatic solid cancers, providing a preliminary understanding of the antitumor activity and safety profile of this combination. The principal trials in this area are summarized in Table 4.a. The side effect profiles of combination radiation therapy and CPIs were generally considered safe and tolerable, and mirrored those seen with CPIs alone, although with the addition of anticipated local toxicity from radiation (see Section 4.6.1 and Table 4.b for additional details). As detailed in Table 4.a, a wide range of stereotactic radiation regimens have been utilized in these studies (20–60 gray [Gy] in 3–5 fractions, given before or concurrently with CPI), with no clear superiority of one schedule over another. The majority of the studies listed in Table 4.a showed a modest overall response rate (ORR) (8%-36%), but only two of these trials were randomized prospective trials comparing CPI alone to CPI with stereotactic radiation therapy (24 Gy or 27 Gy given over 3 fractions (McBride et al. 2018; Theelen et al. 2019)). Unfortunately, neither of these randomized controlled trials produced statistically significant results, failing to clarify the optimally immunogenic dose/schedule for stereotactic radiation in this setting.

Table 4.a Radiation Therapy in Combination With CPI: Overall Antitumor Activity and Abscopal Responses

Drug	Tumor	Modality, Dose (Fractions)	Order Given	ORR (overall)	ORR (abscopal)	PFS (m)	OS (m)	Reference
PD-1 (Pembrolizumab)	NSCLC	SBRT, 24 Gy (3)	Sequential (SBRT->PD-1)	36% (vs 18%)	-	6.6 vs 1.9	15.9 vs 7.6	(Theelen et al. 2019)
PD-1 (Pembrolizumab)	Multiple	SBRT, 30-50 Gy (3-5)	Sequential (SBRT->PD-1)	13%	27%	3.1	9.6	(Luke et al. 2018)
PD-1 (Pembrolizumab)	NSCLC	Radiation, multiple	Sequential (RT->PD-1)	-	-	4.4 vs 2.1	10.7 vs 5.3	(Shaverdian et al. 2017)
PD-1 (Pembrolizumab)	Bladder	SBRT, 24 Gy (3)	Concurrent (PD-1->SBRT)	-	44%	-	-	(Sundahl et al. 2019b)
PD-1 (Pembrolizumab)	TNBC	Radiation, 30 Gy (5)	Concurrent	18%	-	-	-	McArthur et al, 2008 (ASCO poster)
PD-1 (Nivolumab)	TNBC	SBRT, 24 Gy (3)	Sequential (SBRT->PD-1)	-	8%	-	-	(Voorwerk et al. 2019)
PD-1 (Nivolumab)	Melanoma	SBRT, 24 Gy (3)	Concurrent (PD-1->SBRT)	-	45%	-	-	(Sundahl et al. 2019a)
PD-1 (Nivolumab)	HNSCC	SBRT, 27 Gy (3)	Concurrent (PD-1->SBRT)	-	-5%	2.4 vs 1.9	10 vs NR	(McBride et al. 2018)
CTLA4 (Ipilimumab)	Melanoma	SBRT, 50 Gy (4)	Sequential (SBRT->CTLA4)	32%	18%	3.8	10.7	(Twyman-Saint Victor et al. 2015)
CTLA4 (Ipilimumab)	Melanoma	SBRT, 20-50 Gy (1-15)	Concurrent (CTLA4->SBRT)	27%	-	-	-	(Hiniker et al. 2016)
CTLA4 (Ipilimumab)	Multiple	SBRT, 50 Gy (4)	Concurrent or Sequential (CTLA4->SBRT)	-	10%	3.2	10.2	(Tang et al. 2017)
CTLA4 (Ipilimumab)	NSCLC	SBRT, 30 Gy (3-5)	Concurrent	-	18%	3.8	7.4	(Formenti et al. 2018)

CPI: checkpoint inhibitor; CTLA-4: cytotoxic T lymphocyte–associated antigen-4; Gy: gray; HNSCC: head and neck squamous cell carcinoma; m: month; NSCLC: non-small–cell lung cancer; ORR: overall response rate; OS: overall survival; PD-1: programmed cell death protein 1; PFS: progression-free survival; RT: radiation therapy; SBRT: stereotactic body radiation therapy; TNBC: triple negative breast cancer.

4.4 TAK-676 Nonclinical Background

TAK-676 is a synthetic CDN exhibiting highly selective binding and activation of STING. TAK-676 is thought to modulate the innate immune system within the TME through

STING-mediated activation of IRF3, leading to the production of proinflammatory cytokines, such as [redacted] and type I IFNs, and chemoattractant chemokines, such as [redacted]. Type I IFNs are potent immunomodulatory molecules that modulate the activity of innate immune effector cells and promote DC maturation and antigen presentation to T cells, propagating an adaptive immune response, as outlined in Section 4.1 above. The STING pathway has been shown to play a key role in host defense against pathogens both via induction of robust innate immunity and as a bridge between the innate and adaptive immune responses. In mouse syngeneic models (CT26 colorectal cancer, B16F10 melanoma, A20 lymphoma), intravenous (IV) administration of TAK-676 elicits antitumor immunity and demonstrates significant antitumor effect. Moreover, in such models, TAK-676 has also shown synergistic antitumor properties in combination with anti-programmed cell death protein 1 (anti-PD-1) antibodies.

4.4.1 Nonclinical Pharmacology

4.4.1.1 Nonclinical Pharmacology of TAK-676

TAK-676 is a CDN agonist exhibiting highly selective binding and activation of STING proteins from various species, including mouse, rat, monkey, and human. TAK-676 binds to all 4 STING orthologs with dissociation constants estimated to be ≤0.010, 0.008, 0.011, and 0.027 μM for the mouse, rat, cynomolgus monkey, and human STING orthologs, respectively. The agonistic effect of TAK-676 was also evaluated in an in vitro DC activation assay, which identified that TAK-676–mediated STING agonism elicits both mouse and human DC activation. In addition, the sensitivity of CD4 and CD8 T cells and NK cells to TAK-676–induced activation was estimated to be similar in human whole blood. TAK-676 induced the production of [redacted] both in vitro in whole blood taken from A20 tumor-bearing BALB/c mice, as well as in plasma samples isolated from TAK-676–treated A20 tumor-bearing BALB/c mice. [redacted] was the most sensitive cytokine detected.

TAK-676 showed statistically significant in vivo antitumor activity against CT26WT tumors in BALB/c mice (syngeneic colon carcinoma model) when dosed at 1 or 2 mg/kg either once every 3 days (Q3D) or once every 5 days; there were multiple complete regressions. A subset of mice with TAK-676 induced complete regressions were rechallenged using CT26WT cells with and without inhibition of type I IFN signaling mediated by administration of an anti-IFNAR1 antibody. In this rechallenge study, newly injected CT26 cells were unable to form tumors in the mice with functional type I IFN signaling. In contrast, new CT26 tumors did grow in the mice treated with the anti-IFNAR1 antibody. This study indicated that TAK-676 promotes the induction of long-term antitumor immunity in a type I IFN-dependent manner.

Changes in messenger RNA (mRNA) expression of IFN-β1 over time were determined after TAK-676 administration in mice with CT26WT tumors by in situ hybridization. The maximum IFN-β1 mRNA expression was observed at 3 and 6 hours after TAK-676 administration, within the tumor and the stromal regions, respectively. This suggests that the STING pathway was activated in both compartments by TAK-676.

In vivo antitumor activity of TAK-676 was also assessed in A20 tumors in BALB/c mice (syngeneic murine B Cell lymphoma model). TAK-676 exhibited statistically significant ($p<0.001$) antitumor activity against the A20 tumor at both 1 and 2 mg/kg dose levels on a Q3D × 3 schedule. In 1 of 10 mice, complete regression was observed at 1 mg/kg, and in 2 of 10 mice, complete regressions were observed at 2 mg/kg.

The effect of TAK-676 on immune cell populations was evaluated in tumor-draining lymph nodes of C57BL/6J mice bearing B16F10 tumors (syngeneic murine melanoma model) by flow cytometry. The results demonstrated DC activation within the lymph nodes. In addition to DC activation, T cell expansion and acquisition of effector T cell functions were also observed in both the TME and local tumor-associated lymphoid tissue.

4.4.1.2 Nonclinical Pharmacology of TAK-676 and CPIs

Compelling nonclinical data also support the potential of a systemic STING agonist when administered in combination with systemic CPIs. In the A20 syngeneic model, the combination of TAK-676 (0.25 to 0.5 mg/kg; Q3D × 3) and an anti–mouse PD-1 blocking antibody (10 mg/kg; Q3D × 3 followed by once per week [QW] × 3) synergistically suppressed tumor growth. Nonclinical synergy between STING agonism and CPIs has also been reported for other STING agonists (Ager et al. 2017; Ghaffari et al. 2018; Kinkead et al. 2018). The combination of TAK-676 with an anti–mouse PD-1 antibody in tumor-bearing mouse models did not result in synergistic toxicity, as assessed by increased or mortality enhanced body weight loss (BWL) when compared with TAK-676 alone. Additional details about the nonclinical pharmacology of TAK-676 can be found in the IB.

4.4.1.3 Nonclinical Pharmacology of TAK-676 With or Without CPI With Radiation Therapy

Nonclinical evidence for the added benefit of STING agonism in combination with radiation has been generated in the EMT6 murine breast carcinoma model using 2 distinct regimens of radiation treatment: 10 Gy × 1 delivered by focal beam radiation with manual positioning of tumor in the radiation field, and 8 Gy × 3 delivered by focal beam radiation with volumetric image guidance from computed tomography (CT) scans.

In the studies utilizing 10 Gy × 1 delivered by focal beam radiation, combination benefit with TAK-676 was demonstrated on several treatment schedules in 2 studies. In the first study, TAK-676 (1 mg/kg) was administered Q3D × 3, and 10 Gy radiation was delivered in a single fraction, starting 2 days before, on the same day, or 2 days after initiation of TAK-676 dosing. In each case, the combination of TAK-676 and 10 Gy radiation had an additive effect on growth inhibition of irradiated tumors compared with the effect of TAK-676 or radiation alone. A second study in EMT6 tumor-bearing mice also demonstrated additive effects of TAK-676 and radiation on growth inhibition of irradiated tumors (10 Gy radiation delivered 1 or 2 days before, on the same day, or 1 or 2 days after initiation of TAK-676 on a Q3D × 3 schedule). In the first study, the combination was well tolerated with no treatment-related mortality, and BWL was within acceptable limits for all groups. However, in the second study, 2 of 50 mice receiving 10 Gy × 1 radiation and 1 mg/kg TAK-676 were removed from the study due to BWL. Compared with these studies in EMT6 tumor-bearing mice, a study in A20-tumor bearing mice resulted in more BWL

and removal of 9/30 animals receiving 10 Gy × 1 radiation and TAK-676, and 1/30 animals receiving 10 Gy × 1 radiation alone. Overall, the percentage of BWL associated with 10 Gy radiation given as monotherapy was greater in A20-tumor bearing mice than in EMT6 tumor-bearing mice, which may explain the greater BWL with the radiation and TAK-676 combination in A20 tumor-bearing mice. Additional details on these studies are described in Section 4.6.5.

TAK-676 has also been evaluated in the EMT6 model in combination with a fractionated 8 Gy × 3 regimen delivered by 3D volumetric image guidance for target localization and dose delivery, minimizing exposure to nontargeted tissues and organs and allowing a higher total dose to be delivered to the tumor. In this study radiation was administered once daily for 3 consecutive days (QD×3), followed 48 hours later by TAK-676 dosed at 1 mg/kg on a Q3D×3 schedule. Additional groups received radiation on the same schedule, followed 48 hours later by TAK-676 at 0.25 or 1 mg/kg Q3D×3 along with an anti-PD-1 antibody dosed Q3D×3 on the same days as TAK-676. For comparison, the study included groups to test the single agent effects of 8 Gy × 3 radiation, anti-PD-1 antibody, and TAK-676 at 0.25 mg/kg and 1 mg/kg, and a group that received 8 Gy × 3 followed by anti-PD-1. In this experiment, the combination of radiation with TAK-676 at 1 mg/kg, with or without anti-PD-1 antibody, resulted in tumor-free survivors in 5/8 and 6/8 mice, respectively, which persisted until at least day 64 following treatment initiation. In mice treated with TAK-676 at the lower dose of 0.25 mg/kg in combination with radiation and anti-PD-1, 1/8 mice exhibited tumor-free survival until at least Day 64. In contrast, no tumor-free survivors were observed in the groups treated with radiation alone, anti-PD-1 alone, TAK-676 alone, or radiation with anti-PD-1. All treatments were tolerated with no treatment-related deaths and maximum mean BWL <10% in all groups.

4.4.1.3.1 Safety Pharmacology

The potential effects of TAK-676 on the central nervous, cardiovascular, and respiratory systems were determined as part of the Good Laboratory Practice (GLP)-compliant 2-week repeat-dose toxicology studies in rats and monkeys (where TAK-676 was administered twice weekly [BIW] on Days 1, 4, 8, 11, and 15) and in in vitro assays. In the in vitro assays, there were no cardiovascular liabilities identified for TAK-676 at concentrations up to 30 µM based on results from a human ether-à-go-go–related gene (hERG) assay or a human stem cell–cardiomyocyte calcium transient proarrhythmia assay. In addition, in the GLP-compliant 2-week repeat-dose studies there were no TAK-676–related changes in electrocardiogram (ECG) or heart rate in monkeys at doses up to 0.13 mg/kg (slow bolus) and no microscopic findings in the heart, brain, or spinal cord at doses up to 40 mg/kg (slow bolus or infusion) in rats and up to 0.13 mg/kg (slow bolus or infusion) in monkeys. Several clinical signs related to the nervous system were observed at nontolerated doses in the GLP-compliant 2-week repeat-dose studies and were considered secondary to the clinical condition of the animals. The lung was identified as the primary target organ in monkeys, with findings of [redacted] at

0.13 mg/kg. These findings were not observed in monkeys at ≤0.06 mg/kg and were not observed in rats.

4.4.2 Nonclinical Pharmacokinetics

The absorption, distribution, metabolism, and elimination properties of TAK-676 were characterized both in vitro and in vivo in BALB/c mice bearing A20 syngeneic tumors, Sprague Dawley rats, and cynomolgus monkeys after IV dosing. TAK-676 had high clearance in rats and moderate clearance in monkeys. The volume of distribution at steady state after IV administration (V_{ss}) of TAK-676 was moderate in rats and monkeys. TAK-676 showed little to no partitioning into red blood cells (RBCs). TAK-676 protein binding was low in mouse, dog, monkey, and human matrices and was moderate in rat plasma. TAK-676 had low clearance in hepatocytes and was stable in serum isolated from mice, rats, dogs, monkeys, and humans. No unique human metabolite was observed in the in vitro hepatocytes and serum incubations. TAK-676 was mainly eliminated as parent compound in bile duct–cannulated rats and monkeys, and the contribution of metabolism to the clearance pathway in bile duct–cannulated monkeys was considered minimal. TAK-676 has low cellular permeability in human colonic adenocarcinoma cells.

TAK-676 is a substrate for organic anion-transporting polypeptide (OATP) 1B1, OATP1B3, and multidrug resistance associated protein 2 but is not likely a substrate for P-glycoprotein and breast cancer resistance protein. While the OATP inhibitors may affect plasma drug levels of TAK-676, the impact of OATP1B1 polymorphism on TAK-676 exposure is anticipated to be low. Nevertheless, as a precautionary measure, concomitant use of clinical inhibitors of OATP1B1 and OATP1B3 should be avoided before the first dose of study drug(s) and during the study.

The drug-drug interaction (DDI) potential of TAK-676 with cytochrome P-450 (CYP) inhibitors or inducers is unlikely because the contribution of metabolism to the clearance pathway is anticipated to be minimal. The DDIs of TAK-676 as CYP or transporter inhibitors or inducers is unlikely at the currently predicted dose. TAK-676 was projected to have moderate plasma clearance (0.562 L/h/kg), moderate Vss (0.376 L/kg), and short plasma half-life (0.85 h) in humans based on the allometric scaling from monkeys. Additional details about the nonclinical pharmacokinetics (PK) of TAK-676 can be found in the IB.

4.4.3 Nonclinical Toxicology

A battery of nonclinical studies, including in vitro studies for off-target liabilities, genotoxicity, cytokine release in human whole blood, species selection, liver toxicity, and phototoxicity; and in vivo studies (up to 2 weeks in duration) in rats and monkeys (including safety pharmacology endpoints), have been completed to assess the potential toxicologic profile of TAK-676. Sprague Dawley rats and cynomolgus monkeys were selected as relevant toxicology species, as TAK-676 has been shown to activate the STING pathway in these species. IV bolus administration was selected for the toxicology program, as it was considered the best opportunity for observing potential toxicities, including cytokine release syndrome (CRS) if it were to occur. In addition, to proactively evaluate any potential for injection site findings, which may occur with longer exposures of TAK-676 at the injection site (ie, if infusional dosing was used in the clinical

studies), an IV infusion arm was added to the GLP-compliant toxicity studies at the highest doses tested in both rats (40 mg/kg; approximately 10-minute infusion) and monkeys (0.13 mg/kg; approximately 30- and 10-minute infusion in males and approximately 10-minute infusion in females). On the basis of toxicity occurring at a lower nominal dose, with corresponding lower systemic exposures in monkeys compared with rats, the monkey is considered more sensitive than rat as a toxicology species. Pivotal toxicity studies in rats and monkeys (BIW administration for 2-week duration; 5 total doses) were conducted in compliance with GLP Regulations for Nonclinical Laboratory Studies of the United States (US) Food and Drug Administration (FDA) Code of Federal Regulation (CFR), Title 21, Part 58 (21 CFR Part 58) and Organisation for Economic Cooperation and Development Principles of GLP, where applicable. Several studies were exploratory in nature and, although were not conducted in full compliance with all aspects of GLP regulations, are considered scientifically reliable, as they follow standard work practices and standard operating procedures.

4.4.3.1 Summary of Nonclinical Toxicology

TAK-676 was not mutagenic, clastogenic, or phototoxic as evaluated in in vitro assays and has a low risk for off-target, central nervous system, cardiovascular, or hepatotoxic liabilities. Increases in [redacted] levels were observed in an in vitro human cytokine release assay in whole blood and suggest a potential TAK-676–related risk for CRS and inflammatory- and immune-related toxicities in humans.

4.4.3.2 Observed Toxicity in Rats

TAK-676 was well tolerated in 2 exploratory single-dose studies in Sprague Dawley rats at doses up to 33.6 mg/kg (IV bolus) and in an exploratory intermittent repeat-dose toxicity study in rats (BIW for 4 doses or QW for 3 doses) at doses up to 4.1 mg/kg (IV bolus). In the GLP repeat-dose toxicity study in rats, TAK-676 was administered IV BIW (5 total doses) at 10, 20, and 40 mg/kg via bolus (approximately 1-2 minutes). In addition, IV infusion arms, where rats were administered either vehicle (50 mM citrate buffer, 0.5% sodium chloride in sterile water for injection) or TAK-676 at 40 mg/kg BIW (over approximately 10 minutes using an indwelling catheter), were also included to evaluate the potential for injection site findings, which may occur with longer exposures of TAK-676 at the injection site. The tolerability of TAK-676 decreased with repeated administration, causing removal of animals earlier at 40 mg/kg (slow bolus and infusion) on Days 4 through 11 (animals received 2 to 4 doses) and on Day 15 at 10 and 20 mg/kg (after the 5th dose). In dead and/or moribund animals, TAK-676–related decreases in body weight and weight gain were observed that correlated with decreases in food consumption and clinical observations of feces changes (loose, soft, liquid, or mucoid); other observed signs included evidence of poor grooming (yellow and/or brown stained, ungroomed, or erected fur); weakness, decreased activity, hunched posture, prostrate and/or lateral recumbency; cold to touch; generalized pale skin; bilateral pale eyeballs; and/or partially closed eye(s). Clinical pathology evaluation reflected an acute phase response (increases in neutrophil counts, alpha-2-macroglobulin, and triglycerides, and decreases in albumin and cholesterol). Additionally, decreases in lymphocytes, leukocytes, platelets, and red cell counts, and [redacted]

[redacted] and liver enzymes were noted. The predominant microscopic finding at all doses was single-cell necrosis across multiple organs that was most consistent with death of lymphocytes and leukocytes. Additional targets included bone marrow, lymph nodes, thymus, gastrointestinal tract (GIT), hematopoietic system (spleen), soleus muscle, and liver (with supportive clinical pathology changes). TAK-676–related changes in serum cytokines included generally dose-dependent increases at ≥10 mg/kg in [redacted] and keratinocyte chemoattractant-human growth-regulated oncogene, and at ≥20 mg/kg in IL-1β and IL-10. Except for those noted in the liver, findings were generally reversible following a 2-week recovery period. Based on these findings, the severely toxic dose in 10% of rats was 10 mg/kg. At this dose, the [redacted] on Day 15. Compared with the projected human C_{max} and AUC (area under the concentration-time curve) values at the starting dose of 0.2 mg [redacted], exposure margins were 1932× and 477×, respectively. Injection site findings (minimal to mild vascular necrosis) observed in the exploratory intermittent repeat-dose toxicity study in rats were not observed in the GLP repeat-dose toxicity study.

4.4.3.3 Observed Toxicity in Monkeys

In a single-dose exploratory study in monkeys, TAK-676 at ≤0.08 mg/kg (IV slow bolus) was clinically tolerated. At doses of 0.2 and 0.8 mg/kg, TAK-676 was not tolerated and resulted in the death (2 at 0.8 mg/kg) or early removal (1 each at 0.2 and 0.8 mg/kg) of monkeys on Day 2. Clinical observations in these animals on Day 2 included decreased activity, dehydration, pain with abdominal palpation, limited use of limbs, lying on side, agonal breathing, pale skin, ataxia, vomitus, hunched posture, non-responsiveness, and/or hypothermia. Macroscopic findings at necropsy in the monkeys removed early included [redacted], which correlated microscopically with [redacted]. Transient, dose-dependent increases in [redacted], were observed and were generally higher in monkeys that died or were removed early.

In an exploratory intermittent repeat-dose toxicity study in monkeys, TAK-676 was administered via IV bolus injection Q3D at 0, 0.01, 0.03, 0.08 mg/kg for 4 doses or QW for 3 doses at 0.13 mg/kg. All monkeys survived and TAK-676 was clinically tolerated at all doses. TAK-676-related findings were limited to clinical pathology changes that were consistent with an acute phase response and transient increases in [redacted]. There were no macroscopic or microscopic findings (including no lung findings) at any dose. Based on these findings, the maximum tolerated dose (MTD) and no-observed-adverse-effect level (NOAEL) were considered to be 0.08 mg/kg Q3D and 0.13 mg/kg QW.

In the GLP repeat-dose toxicity study in monkeys, TAK-676 was administered IV BIW (5 total doses) at 0.025, 0.06, and 0.13 mg/kg via slow bolus (approximately 1 minute). In addition, IV infusion arms, where monkeys were administered either vehicle (50 mM citrate buffer, 0.5% sodium chloride in sterile water for injection) or TAK-676 at 0.13 mg/kg BIW (approximately 30 and 10 minutes for males and 10 minutes for females; 1 to 3 doses total), were

also included to evaluate the potential for injection site findings, which may occur with longer exposures of TAK-676 at the injection site. Due to clinical intolerability by Day 2 at 0.13 mg/kg with the 30-minute infusion in male monkeys (males administered TAK-676 before females in a staggered study design), in combination with a perceived tolerability in the bolus dosing group at the same dose level through Day 2, including a lack of clinical signs or body temperature changes indicative of CRS in either the bolus or infusion group through Day 2, the duration of the infusion was reduced from 30 minutes to 10 minutes in male monkeys on Day 8 following a dosing holiday on Day 4. In addition, a 10-minute infusion was used for the female monkeys starting on Day 1.

TAK-676 was clinically well tolerated at ≤0.06 mg/kg, with all monkeys surviving to their scheduled necropsies. At 0.13 mg/kg (slow bolus and 10- and 30-minute infusions), TAK-676 was not tolerated and resulted in mortality and/or early removal of monkeys. Administration of TAK-676 via slow bolus at 0.13 mg/kg resulted in the removal of 1 of 12 monkeys from the study on Day 6 due to clinical signs of hunched posture, ataxia, uncoordinated movements (with limited usage of right forelimb), pale mucous membranes, and a decreased body temperature on the removal day. Administration of TAK-676 at 0.13 mg/kg via 30- or 10-minute infusion resulted in the death (2 of 12 monkeys; 1 male and 1 female) or early removal (3 of 12; 2 males and 1 female) of monkeys due to poor clinical condition on Day 2. Clinical signs in moribund monkeys (on Day 2 only) included lying on side, difficulty breathing, cold to touch, pale gums/mucous membranes, [redacted]. Although clinical signs of distress were not observed in the 2 monkeys found dead on Day 2, findings at necropsy (described below) suggest that clinical signs likely preceded death and were possibly similar in nature to those described for moribund monkeys on Day 2. Despite changes in the length of infusion, acute intolerability persisted following the initiation of dosing in females, and all remaining animals in the infusion group were removed from study approximately 6 hours after a final dose on Days 4 (females), 8 (females), or 11 (males).

A review of systemic exposures in the infusion and slow bolus groups revealed a slightly higher mean AUC_{24} (approximately 1.6-fold) in the infusion group compared with the bolus group. In addition, retrospective exposure (AUC_{24})-toxicity regression modeling suggests that the toxicity is related to the AUC_{24} in individual animals and is independent of route of administration. Collectively, these data suggest that slightly higher individual AUC_{24} in the infusion group accounts for the perceived differences in tolerability between the bolus and infusion dosing groups.

Clinical pathology findings consistent with an acute phase response and transient increases in [redacted] at all doses were observed. Transient, TAK-676–related increases in [redacted] were generally observed in monkeys assigned to the 0.13 mg/kg bolus and infusion groups on Day 1. Because increases in [redacted] were observed in some monkeys that survived past Day 2 but not in 1 monkey that died, the relationship between the increases in [redacted] and the observed toxicity at 0.13 mg/kg is currently unclear. The target organ toxicity consisted of lung changes, including [redacted] in 1 female that survived to scheduled necropsy on Day 16 at 0.13 mg/kg (slow bolus), and, in the animals that died or were removed early from the study, [redacted] that correlated with clinical signs [redacted]

[redacted]. The lung findings were considered to be consistent with [redacted] and were considered directly related to the poor clinical tolerability and early mortality at the 0.13 mg/kg dose. These findings were not observed in monkeys evaluated following the 2-week postdose period. There were no microscopic findings in monkeys at ≤0.06 mg/kg and no injections site findings at any dose. Based on these findings, 0.06 mg/kg was both the highest nonseverely toxic dose (HNSTD) and the NOAEL ([redacted], respectively, on Day 15). Compared with the projected human C_{max} and AUC values at the starting dose of 0.2 mg [redacted], exposure margins at this dose were 14.6× and 5.6×, respectively.

4.4.3.4 Nonclinical Toxicology of TAK-676 in Combination With Radiation or Anti-PD-1 Antibody

Nonclinical toxicology studies evaluating the combination of TAK-676 and radiation or pembrolizumab, a monoclonal antibody that binds to the human PD-1 receptor and blocks the interaction of PD-1 with PD-1 ligands, or another anti-PD-1 agent were not conducted and are not warranted per ICH S9. The tolerability of TAK-676 in combination with radiation or anti-mouse-PD-1 antibody was assessed in pharmacology studies in tumor-bearing mice and are described in Sections 4.6.4 and Section 4.6.5.

Additional details about the nonclinical toxicology of TAK-676 can be found in the IB.

4.5 Rationale for the Proposed Study

Study TAK-676-1003 intends to evaluate the safety, tolerability and preliminary efficacy of radiation therapy in combination with pembrolizumab and TAK-676. This therapeutic approach seeks to take advantage of the well-described immune-modulating effects of radiation therapy, which results in the generation of cytosolic DNA and extracellular release of tumor antigens associated with radiation-induced cell death. The presence of cytosolic DNA in both the tumor and immune cells, in turn, activates the cGAS/cAMP/STING pathway, leading to increased downstream induction of type I IFNs. These changes lead to an increase in tumor antigen uptake and cross-presentation by DCs, with associated cross-priming of tumor-specific T cells, leading to adaptive immunity.

Evidence exists that heightened STING activation may result in an immunosuppressive phenotype, with increased expression of PD-L1 on tumor cells and PD-1 on lymphocytes, as well as tumor infiltration of immune-suppressive cells, such as regulatory T cells and myeloid-derived suppressor cells. Administration of an anti-PD-1 or anti-PD-L1 antibody along with radiation may attenuate this inhibitory state, leading to an increase in immune-mediated tumor cell death, both locally at the site of irradiation and systemically in nonirradiated tumors (ie, abscopal effect). The addition of the STING agonist TAK-676 to this combination seeks to further heighten the immune response elicited by radiation therapy by increasing the STING-mediated type-I IFN response, further stimulating formation of a T Cell-mediated adaptive anti-tumor effect.

4.5.1 Rationale for the Selected Patient Population

This study will enroll adult patients with histologically confirmed advanced or metastatic solid tumors who have received or been offered all established standard of care (SOC) treatment options for which they are eligible and whose disease previously progressed while on CPIs in a prior line of therapy. This patient population represents a traditional cohort for phase 1 studies containing a new regimen without a well-defined, biomarker-driven or tumor-specific target. This patient population often has the most favorable balance between patient risk and benefit for a phase 1 study. During dose escalation, this study will collect tumor tissue and blood samples to identify potential pharmacodynamic biomarkers to support future patient selection or enrichment.

This trial specifically recruits patients who are refractory to or have developed resistance to anti-PD-1/anti-PD-L1 therapies. There is preclinical evidence that the addition of a STING agonist may reverse the mechanisms of resistance in tumors with prior exposure to anti-PD-1/anti-PD-L1 therapies. Additionally, mouse models of TAK-676 combined with radiation demonstrate an added benefit from this combination. Thus, while it is unknown whether the addition of radiation therapy to the combination of anti-PD-1 antibody and TAK-676 will provide meaningful clinical benefit, this regimen represents a promising approach to addressing CPI recalcitrance. Tumor responses in this group of patients may provide early insight into the clinical benefit produced by the combination of TAK-676 and pembrolizumab following radiation therapy for patients in which CPI alone is not sufficient to drive or maintain a sustained clinical response.

4.5.1.1 NSCLC

NSCLC is the leading cause of cancer mortality in the US with an estimated 140,000 patients dying of the disease this year, which is greater than the number of cancer mortalities from colon, breast, and prostate cancer combined (Siegel et al. 2019). The majority of NSCLC patients present with metastatic disease. Recently, immunotherapy with or without chemotherapy (depending on PD-L1 expression) has become the proven preferred first or second-line systemic therapy in patients without actionable tumor driver mutations (Borghaei et al. 2019; Gandhi et al. 2018; Reck et al. 2019). Similarly, radiation therapy plays an important role in the management of advanced NSCLC, whether in early or late-stage disease. Unfortunately, despite the overall success of ICB, the vast majority of patients eventually develop resistance to immunotherapy and have disease progression.

4.5.1.2 TNBC

TNBC represents up to 20% of all breast cancers and is defined by a lack of estrogen and progesterone receptor expression and the absence of human epidermal growth factor receptor 2 overexpression and/or amplification (Foulkes et al. 2010). The lack of hormone expression precludes the use of endocrine therapy, the backbone of treatment in metastatic hormone receptor-positive breast cancer. Thus, chemotherapy is the traditional mainstay of treatment in metastatic TNBC, with short-lived responses and limited prognosis in this patient population (Li et al. 2017). There has thus been much excitement about the potential use of ICB in TNBC (Adams et al. 2019a). Initial studies have shown objective response rates, many with durability, with ICB monotherapy of up to 23% in first-line patients with PD-L1 positive tumors (Adams et al. 2019b).

Notably, the IMPASSION 130 phase 3 trial evaluated nab-paclitaxel +/- atezolizumab in 902 previously untreated metastatic TNBC and showed improved progression-free survival (PFS) of 7.2 months in the atezolizumab arm compared with 5.5 months in the placebo arm ($p < 0.002$); median overall survival was 21.3 months in the atezolizumab arm versus 17.6 months with placebo ($p = 0.08$), and an even greater benefit was seen in the PD-L1-positive population (Schmid et al. 2018). Although this study initially received accelerated approval for first-line use in patients with metastatic TNBC and positive PD-L1 expression levels, this approval has since been withdrawn, further highlighting the challenges in treating this type of breast cancer. In contrast, recent results from the phase 3 Keynote-355 trial showed a significant increase in PFS (9.7 vs 5.6 months; $p = 0.0012$) for patients with metastatic TNBC and a combined positive score ≥10 who were treated with pembrolizumab plus chemotherapy (nab-paclitaxel, paclitaxel, or carboplatin/gemcitabine) versus chemotherapy alone. On the basis of this study, pembrolizumab in combination with chemotherapy for the treatment of patients with locally recurrent unresectable or metastatic TNBC whose tumors express PD-L1 (CPS ≥10) has been granted accelerated approval. Currently, once a patient progresses on either atezolizumab plus nab-paclitaxel or pembrolizumab plus chemotherapy, they may have the option of being treated in a later line with the antibody drug conjugate, sacituzumab govitecan, but beyond this, the treatment options remain limited.

4.5.1.3 SCCHN

In 2020, an estimated 53,260 new cancers of the oral cavity and pharynx are expected to be diagnosed, and 10,750 people will die of the disease. Key risk factors for SCCHN are smoking, alcohol consumption, human papillomavirus, and Epstein-Barr virus. Only 29% of patients are diagnosed with localized disease that is curable by surgery alone. SCCHN has a predominantly locoregional pattern of recurrence, with only 25% of patients presenting with distant metastases at recurrence, and this mostly to the lungs. Chemoradiation therapy plays a cornerstone role in the treatment of locally advanced SCCHN both in a first-line definitive or postoperative setting (National Comprehensive Cancer Network 2020; Quon et al. 2017; Sher et al. 2017). More recently, pembrolizumab was FDA-approved in the first-line setting for the treatment of metastatic or unresectable SCCHN as monotherapy for patients with positive PD-L1 expression and in combination with platinum and fluorouracil in patients whose tumors are PD-L1 negative. Nivolumab is also approved for use in the second-line setting after progression on a platinum-containing agent. Although the role of pembrolizumab combined with radiation therapy is currently being studied (Yu and Lee 2019), there is an unmet clinical need for those patients who have progressive disease (PD) while on CPI and chemotherapy.

4.5.2 Rationale for Dose of Radiation Therapy

As noted in Section 4.2, multiple studies have evaluated the combination of radiation therapy and CPIs in metastatic solid malignancies. Dosing regimens in these studies have ranged from 20 to 60 Gy given over 3 to 5 fractions. The ORRs seen in nonirradiated lesions (abscopal effect) in these studies is generally modest, with rates ranging from 8% to 45%. Only two of these studies included randomized controls (McBride et al. 2018; Theelen et al. 2019), and neither of these studies showed statistically significant differences in response rates between the CPI monotherapy and

combined CPI plus stereotactic radiation cohorts. Consequently, it is impossible to definitively state a radiation dose and schedule that is optimally immunogenic in this setting. Nonetheless, three of the most promising trials from Table 4.a utilized a common radiation therapy regimen with 24 Gy given over 3 equal fractions, either concomitantly with CPI (pembrolizumab (Sundahl et al. 2019b) or nivolumab (Sundahl et al. 2019a)) or preceding CPI (pembrolizumab (Theelen et al. 2019)). Two studies by Sundahl et al. evaluated the concomitant use of stereotactic body radiation therapy (SBRT) of 8 Gy × 3 with either pembrolizumab or nivolumab (RT given before the second cycle of CPI) in bladder cancer and melanoma, respectively (Sundahl et al. 2019a; Sundahl et al. 2019b). Although small, each of these trials showed an ORR in the nonirradiated lesions of approximately 45%. Additionally, the randomized phase 2 PEMBRO-RT trial noted above evaluated SBRT at 8 Gy × 3 followed by pembrolizumab in advanced NSCLC. While the results of this study did not reach statistical significance, there was an observed improvement in ORR in nonirradiated lesions (36% vs 18%, p = 0.07), median PFS (6.6 vs 1.9 months, p = 0.19), and mOS (15.9 vs 7.6 months, p = 0.16), suggesting the need for further exploration of this approach. These results, as well as the tolerable safety profile seen in these and other studies utilizing SBRT at 8 Gy × 3 doses (Table 4.b) (Maity et al. 2018; McBride et al. 2018; Voorwerk et al. 2019), support the use of this dosing schedule as a safe and adequately immunogenic regimen.

Additionally, recent preclinical data appear to favor fractionated radiotherapy (8 Gy × 3) over 20 Gy × 1 due to the ability of the 8 Gy × 3 regimen to generate a level of cytosolic DNA which activates cGAS and STING, resulting in upregulation of the type I IFN pathway and consequent activation of antigen-presenting DCs in the tumor. In contrast, the 20 Gy × 1 regimen upregulates the exonuclease Trex1, leading to cytosolic DNA degradation and preventing the activation of cGAS and its downstream effects (Vanpouille-Box et al. 2017).

4.5.3 Rationale for Proposed Starting Dose of TAK-676

To appropriately balance the potential risk of developing acute adverse events (AEs) from immune stimulation in the cancer patient population and the potential for pharmacologic activity at the starting dose, the maximum recommended starting dose (MRSD) from the GLP-compliant 2-week repeat-dose monkey toxicity study (most sensitive species) and the minimum anticipated biological effect level (MABEL) were considered in selecting the starting dose of TAK-676. Please refer to the IB for additional details.

The dose of 0.2 mg TAK-676 corresponds to the MABEL. Furthermore, the dose of 0.06 mg/kg was considered both the HNSTD and NOAEL in the GLP-compliant 2-week toxicity study in cynomolgus monkeys. Using this dose, the estimated MRSD in humans was 0.19 mg. This estimation was based both on body surface area and AUC calculations and includes the 1/6th safety factor calculation per ICH S9 and is quasi-concordant with the MABEL-based dose calculation.

4.5.3.1 Dose Range

The proposed dose range for TAK-676 in the dose escalation phase of the study is 0.2 to 9.0 mg administered on Days 1, 8, and 15 of every 21-day cycle.

The ongoing TAK-676-1002 first-in-human (FIH) study, where TAK-676 was administered by IV infusion once weekly alone or in combination with pembrolizumab, will provide initial clinical data on the safety and tolerability of TAK-676 + pembrolizumab. In the combination arm of the FIH study, TAK-676 is administered in a dose-escalating fashion (starting at 0.2 mg IV on Days 1, 8, and 15 of a 21-day cycle) along with pembrolizumab at the fixed dose of 200 mg IV every 3 weeks. Safety data was to be obtained from at least 2 dose cohorts of the FIH combination arm before administering TAK-676 with pembrolizumab following radiation therapy in this study. The dose escalation phase of the TAK-676-1002 FIH study is ongoing and no dose-limiting toxicities (DLTs) or preliminary safety/tolerability concerns were observed at the preplanned interim safety data review at 1.2 mg TAK-676 or through 0.8 mg TAK-676 in combination with pembrolizumab, confirming that it is safe to proceed with enrollment in this study at the proposed starting dose level. Dose escalation in the FIH study is ongoing at higher dose levels, and the highest proposed dose in this study will not exceed the highest dose level determined to be safe in the FIH study.

4.5.4 Rationale for Paired Biopsies and Blood Draws

Tumor and blood tissues will be collected to assess changes over time in the tumor and the innate and adaptive immune response following treatment with radiation, pembrolizumab, and TAK-676.

4.5.4.1 Tumor Biopsies

Tumor biopsies of nonirradiated tumors at baseline and on-treatment will be collected to determine modulation of the innate and adaptive immune response in the TME, including evaluation of T cell diversity. These samples may also be evaluated for tumor mutational burden and to determine treatment effects in cancer cells and immune cells.

Induction of an innate and/or adaptive immune response in the tumor will be assessed by measuring levels of mRNA and protein using RNA sequencing and slide-based assays for evaluation of levels of tumor infiltrating lymphocytes and activation states of innate and adaptive immune cell subsets. Additional analyses related to resistance, response or disease characterization may be done on collected biopsies.

To minimize the risk of performing invasive procedures on patients at TAK-676 dose levels with no measurable pharmacodynamic activity, paired biopsies of nonirradiated tumors will be required only for patients on dose levels of TAK-676 that have demonstrated evidence of pharmacodynamic activity in peripheral blood (refer Section 9.4.15.1). Additionally, once the tumor biopsies are mandated, safely accessible paired biopsies will be collected from all patients during dose escalation and additional patients are expected to be added if an expansion phase is supported, to achieve a greater than 0.8 predicted posterior probability of increased immune infiltration within the tumor (refer to Appendix K). However, paired biopsies are highly desired and strongly encouraged in all patients, regardless of dose level, if the lesions are deemed safely accessible. The specific anatomical location of the biopsy before resection must be specified.

4.5.4.2 Blood

Blood samples will be collected to demonstrate STING pathway activation and activation of an innate and/or adaptive immune response. This will be assessed by measuring levels of select plasma chemokines and cytokines (eg, IP-10), detection of a STING agonism/type I IFN gene signature, changes in the number, percentage, or activation state of immune cell subsets in the blood, and changes in T cell clonality.

4.5.5 Rationale for PK Assessments

Serial plasma PK of TAK-676 will be assessed. The PK data collected on these occasions will coincide with pharmacodynamic assessments. PK data in conjunction with pharmacodynamic data can help in the understanding of the PK-pharmacodynamic relationship of TAK-676. Such data may also be useful in building mathematical models to describe the PK-pharmacodynamic and/or PK-safety/efficacy relationship of TAK-676 that can eventually be used to predict the time course of PK and pharmacodynamic effects of TAK-676. This information may also help provide context for safety findings from the study and can be helpful in selecting the recommended phase 2 dose (RP2D) and schedule for TAK-676.

4.6 Potential Risks and Benefits

The potential effects listed below are based on publicly available data on the effects of radiation as well as other clinical studies that have evaluated radiation in combination with pembrolizumab. The potential risks associated with TAK-676 administration are reviewed below and in further detail in the IB.

4.6.1 Potential Risks and Effects of Radiation in Combination with Pembrolizumab

Multiple clinical studies have reported on the safety of the combination of radiation therapy and pembrolizumab (Table 4.b). Overall, the combination was found to be safe with AEs similar to those commonly associated with pembrolizumab. Most of the observed AEs were of Grade 1 and 2, including dermatitis reported in 29% of patients in 1 study. AEs of Grade 3 in severity were reported infrequently, including fatigue, lymphopenia, infection, pneumonitis, infusion-related reaction and weight loss. Rare Grade 4 AEs were observed, including Grade 4 adrenal insufficiency in 1 study.

An increased rate of radiation necrosis in patients with brain metastases from melanoma who received stereotactic radiosurgery (SRS) and CPI was reported. No reports of radiation necrosis were noted in patients with NSCLC, TNBC, or SCCHN receiving radiation therapy in combination with pembrolizumab or other CPIs. In 1 study evaluating pembrolizumab and multisite stereotactic body radiotherapy in 79 patients with advanced solid tumors, toxicity was generally low; however, when toxicity was observed, it appeared to be in the region that was irradiated, which made it difficult to distinguish between toxicity of combination therapy versus radiation alone (Luke et al. 2018). A retrospective review of 133 patients treated with palliative radiotherapy and anti–PD-1 showed a 4% Grade 3 or higher immune-related AE rate, and toxicity was not related to anatomic location of RT (Bang et al. 2017). Additional potential AEs related to

radiation therapy include pain, inflammation, skin ulceration and/or tissue necrosis in the radiation field, and/or localized or systemic infection.

Available data (Table 4.b) indicates that the combination of radiation therapy with various immune CPIs is generally well tolerated.

Table 4.b Trials Showing Safety and Tolerability of Radiation Therapy in Combination With CPI

Phase of Trial	Treatment	Patient Population	Number of Patients	CPI Dose and Radiation Fractionation	Safety	Reference
Phase 1	Pembrolizumab + SBRT	Metastatic solid tumors	24	Pembrolizumab: 6 w before RT RT: 8 Gy × 3 fractions or 17 Gy × 1 fraction	Two irAEs (hypothyroidism and pneumonitis) occurred post radiation to a lung metastasis in the same patient who achieved a CR	(Maity et al. 2018)
Phase 2	Pembrolizumab vs SBRT + Pembrolizumab	Metastatic NSCLC	74	RT: 8 Gy × 3 fractions	No Grade 3 or higher toxicities related to the addition of SBRT	(Theelen et al. 2018)
Phase 2	Pembrolizumab + RT	Metastatic TNBC	17	Pembrolizumab: 200 mg 1-3 d after first RT fraction, and then every 3 weeks until disease progression RT: Total 3000 cGy (600 cGy × 5 fractions) over 5-7 days	Most common Grade 1 to 2 toxicity: dermatitis (29%); Grade 3 pembrolizumab-related AEs (n = 4): fatigue, lymphopenia, and infection	(Ho et al. 2020)
Phase 1	Pembrolizumab + RT	Locoregionally advanced head and neck cancer	8	Pembrolizumab: 200 mg at Weeks -2, 1, 4, and 7 with RT RT: 70 Gy in 35 daily fractions, Weeks 1-7	Grade 4 adrenal insufficiency definitely related to pembrolizumab (n = 1); Grade 3 infusion-related reaction (n = 1); Grade 3 weight loss (n = 1)	(Sacco et al. 2019)

Table 4.b Trials Showing Safety and Tolerability of Radiation Therapy in Combination With CPI

Phase of Trial	Treatment	Patient Population	Number of Patients	CPI Dose and Radiation Fractionation	Safety	Reference
Phase 1/2	Pembrolizumab with and without RT	Metastatic NSCLC	124	Pembrolizumab: 200 mg IV on Day 1 and given every 3 w for up to 16 cycles SBRT: 50 Gy in 4 fractions or 70 Gy in 10 fractions or traditional fractionation: 45 Gy in 15 fractions	Combined-modality arm: Grade 4 (n = 2) and Grade 3 (n = 9) treatment-related toxicities; pembrolizumab arm: no Grade 4 toxicities and Grade 3 toxicities (n = 5)	(Welsh et al. 2019)
Retrospective study to review risk of RN	Stereotactic radiosurgery and CPI (ipilimumab, pembrolizumab, or both)	Metastatic melanoma with brain metastases	137	-	Rate of RN in patients with metastatic melanoma treated with SRS and immunotherapy with either CTLA-4 inhibitor, PD-1 inhibitor, or both is 27% with a median time to RN of 6 months	(Fang et al. 2017)
Retrospective study	Stereotactic radiosurgery and CPI	Brain metastases from melanoma	480	-	Patients who received SRS and CPI had higher rates of symptomatic RN than those who received SRS alone	(Martin et al. 2018)

cGy: centigray; CPI: checkpoint inhibitor; CR: complete response; CTLA-4: cytotoxic T lymphocyte–associated antigen-4; Gy: gray; IV: intravenous(ly); NSCLC: non-small-cell lung cancer; PD-1: programmed cell death protein 1; RN: radiation necrosis; RT: radiation therapy; SBRT: stereotactic body radiation therapy; SRS: stereotactic radiosurgery; TNBC: triple-negative breast cancer.

4.6.2 Potential Risks and Effects of TAK-676 Based on Nonclinical Studies

The potential effects listed below are based on the findings from nonclinical studies with TAK-676 and publicly available data on other CDNs that are currently in clinical development. These events may or may not develop in human patients treated with TAK-676. It is also possible that the administration of TAK-676 during a clinical study will result in AEs that were not observed or predicted from the completed nonclinical studies conducted in animals and/or in studies with other CDNs. Please refer to the IB for additional detail.

In this study, TAK-676 will only be administered at dose levels previously shown to be tolerable in the FIH trial, providing further insight into the side-effect profile of this drug before initiation of the study.

Potential risks identified from nonclinical studies in rats and monkeys are described below.

Pulmonary Vasculature Toxicity/Noncardiogenic Pulmonary Edema

Pulmonary changes in the monkey toxicology studies suggest that TAK-676 could cause pulmonary vascular toxicity considered consistent with vascular leak.

In the single-dose and pivotal repeat-dose studies in monkeys, macroscopic findings were limited to monkeys removed from study early or found dead and included [redacted]. Microscopic findings in the lungs of these monkeys included [redacted]. The findings were considered to be consistent with [redacted] and directly related to the poor clinical tolerability and early mortality at this dose. [redacted] was also observed in 1 monkey in the repeat-dose study that survived to the scheduled necropsy.

CRS

Increased pro-inflammatory cytokines, including [redacted] and [redacted] were observed in an in vitro human whole blood cytokine release assay, indicating a potential risk for CRS.

There were no clinical signs of CRS (increases in body temperature or changes in heart rate [monkeys]) noted in the toxicology studies with rats and monkeys. However, there was clinical pathology evidence of an acute phase response in both species. In addition, increases in [redacted] (rats only) and [redacted] (rats and some monkeys) were observed, but the relationship between the increases in [redacted] and the observed toxicity at 0.13 mg/kg in monkeys is unclear.

These findings raise the possibility of a CRS in the clinical study.

To date, no cases of CRS have been reported with other CDNs following intratumoral administration at doses up to 3 mg per injection.

Immune and Lymphoid System Toxicity

Findings in toxicity studies in rats suggest that lymphoid systemic toxicity could occur in patients treated with TAK-676.

In exploratory studies in rats, [redacted] in Peyer's patches and lymph nodes and [redacted] were noted.

In the repeat-dose (GLP) study in rats, [redacted] were observed and correlated with [redacted] in the thymus, and [redacted] in the spleen (related to decreased [redacted] on hematology). In addition, [redacted] were noted in lymph nodes and thymus that generally correlated with gross observations of [redacted]. Findings included [redacted] that was most consistent with [redacted] across multiple organs (lymph nodes, spleen, gut-associated lymphoid tissue, bronchus-associated lymphoid tissue, and thymus), which was associated with [redacted] in these tissues, and in perivascular and interstitial areas in skeletal muscle (soleus) and epididymis; [redacted] in the portal interstitium of the liver, in the lamina propria and submucosa of the GIT (males only; stomach, duodenum, jejunum, ileum, colon, rectum, and cecum) that was occasionally associated with [redacted], in the bone marrow, and in the perivascular and interstitial areas of the lung; and [redacted] in the cervix. These findings could not be correlated with clinical observations in the animals. Comparable effects were not observed in monkeys. The clinical relevance of these findings is unknown.

Additionally, overactivation of the immune system by STING agonists may lead to autoimmune reactions and diseases.

Because of the risks of lymphoid system toxicity following treatment with TAK-676, regular monitoring of blood counts and peripheral blood immunophenotyping will be carried out in all patients. In addition, all patients will be monitored for timely diagnosis and management of autoimmune endocrinopathies and organ disorders (including collection of thyrotropin, T4, T3, and glucose; assessment of liver and renal functions).

Effects on Skeletal Muscle

In the repeat-dose (GLP) study in rats, [redacted] were noted in the perivascular and interstitial areas of the soleus muscle and were associated with occasional and reversible [redacted] Comparable effects were not observed in monkeys. The clinical relevance of these findings is unknown. Patient creatine phosphokinase (CPK) levels will be monitored according to the study evaluation schedule and on an ad hoc basis as needed.

Effects on GIT

In the repeat-dose (GLP) study in rats' findings included dose-dependent [redacted] in the colon, cecum, and rectum; and discoloration of the stomach, jejunum, and ileum that generally correlated with [redacted] within the lamina propria and submucosa. In addition, feces changes (loose, soft, liquid, or mucoid) and decreases in body weight, body weight gain, and food consumption that required food supplementation were noted in animals found dead or removed from the study early.

The gross and microscopic findings noted in the GIT could not be directly correlated with the clinical observations in the rats. Comparable effects were not observed in monkeys. The clinical relevance of these findings is unknown. Patients will be clinically monitored for gastrointestinal (GI)-related AEs while on study.

Liver Toxicity

Findings of [redacted] with corresponding changes in serum chemistry parameters were observed in the repeat-dose (GLP) study in rats and suggest a potential for liver toxicity in patients treated with TAK-676.

To monitor for this risk in clinical study patients, this study includes standard exclusion criteria for patients with aspartate aminotransferase (AST) and alanine aminotransferase (ALT) >3 times the upper limit of normal (ULN) and total bilirubin >1.5 times the ULN. All patients will have liver function tests monitored regularly for timely diagnosis and management of any observed liver dysfunction.

Bone Marrow Toxicity

Findings of [redacted] in the bone marrow and associated [redacted] on hematology were observed in the repeat-dose (GLP) study in rats and demonstrate the potential for bone marrow toxicity in patients treated with TAK-676.

To monitor for side effects of bone marrow toxicity in patients treated with TAK-676, complete blood counts will be monitored regularly for all patients. If anemia, thrombocytopenia, or neutropenia occur, the TAK-676 dose will be modified according to the guidance in Section 8.4. Prophylactic use of myeloid growth factors should be avoided during the first cycle of dose escalation. Transfusion and use of growth factors as necessary to manage anemia, thrombocytopenia, and neutropenia are permitted, consistent with American Society of Hematology (ASH)/American Society of Clinical Oncology (ASCO) guidelines and institutional recommendation. See Section 8.5 for additional guidance surrounding use of these products.

Vascular Toxicity at the Injection Site

In an exploratory repeat-dose toxicity study in rats, microscopic findings of [redacted] that had an increased incidence and severity in animals administered TAK-676 and was accompanied by [redacted] were noted and suggested that vascular toxicity at the infusion site could occur in patients treated with TAK-676. Injection site changes were not noted in the repeat-dose GLP study in rats or in any monkey studies.

Injection site pain (Grade 3) and skin necrosis (Grade 3) have been reported with other STING agonists administered intratumorally (Harrington et al. 2018).

Patients will be monitored for any discomfort, pain, or swelling at the infusion site. In case of extravasation, infusion should be stopped, and clinical symptoms managed according to institutional standards.

Coagulopathy

Nonadverse [redacted] were noted in the GLP repeat dose rat toxicity study and suggest that a coagulopathy could occur in patients treated with TAK-676.

To minimize the risk of coagulopathy, patients treated with TAK-676 will have their coagulation function monitored according to the study evaluation schedule and on an ad hoc basis as needed.

4.6.3 Potential Risks and Effects Seen With Other STING Agonists

Additional risks have been reported with other STING agonists and are described below. Although these risks were not observed in TAK-676 animal studies, they should be carefully monitored.

- There were no relevant findings from nonclinical toxicology studies indicating a potential for skin-related toxicities. Skin rashes are commonly associated with activation of type I IFN. Patients with congenital STING activating mutations (STING-associated vasculopathy with onset in infancy-like disorder) have skin lesions, including telangiectatic lesions on nose and cheeks and violaceous, scaling atrophic plaques on hands, and vascular inflammation in skin biopsy.
- No cardiovascular liabilities were identified for TAK-676 based on in vitro results from a hERG or human stem cell–cardiomyocyte calcium transient proarrhythmia assay. In addition, there were no changes in qualitative ECG parameters or heart rate in evaluated monkeys following repeated administration of TAK-676 at doses up to 0.13 mg/kg. However, literature data indicate that CDNs can impact hyperpolarization-activated cyclic nucleotide-modulated channels that regulate the rhythmic firing of neurons and cardiac myocytes; thus, potentially affecting cardiac repolarization (Lolicato et al. 2014).

4.6.4 Potential Risks and Effects of TAK-676 in Combination With Pembrolizumab

Nonclinical toxicology studies evaluating the combination of TAK-676 and pembrolizumab were not conducted and are not warranted per ICH S9. Pembrolizumab is a monoclonal antibody that binds to the human PD-1 receptor and blocks the interaction of PD-1 with PD-L1/PD-L2. TAK-676 at 0.25, 0.5, or 1 mg/kg was administered to tumor-bearing mice Q3D (Days 2, 5, and 9) alone or in combination with 10 mg/kg anti-mouse PD-1 antibody (Q3D×3 followed by QW×3). Compared with treatment with TAK-676 alone, synergistic toxicity, as assessed by increased mortality or more significant decreases in body weight was not observed in mouse tumor-bearing models administered TAK-676 in combination with an anti-mouse PD-1 antibody. TAK-676 and pembrolizumab both activate the immune system, so known PD-1 immunotherapy-related toxicities (such as pneumonitis, colitis, hepatitis, dermatitis, infusion-related reactions, endocrinopathies, and nephritis) observed following the administration of pembrolizumab may be enhanced when combined with TAK-676. Details regarding the full safety profile of pembrolizumab can be found in the most recent prescribing information.

4.6.5 Potential Risks and Effects of TAK-676 in Combination With Radiation and Pembrolizumab

Radiation-related AEs (including inflammatory reactions, infections and abscess within the radiation field) may occur or be enhanced when radiation is combined with TAK-676 and pembrolizumab.

Nonclinical toxicology studies evaluating the combination of TAK-676 and radiation were not conducted and are not warranted per ICH S9 .

Nonclinical Pharmacology Studies of Antitumor Activity of TAK-676 in Combination With Radiation in Syngeneic Mouse Models:

Tolerability, as assessed by enhanced mortality and BWL, was monitored in pharmacology studies in tumor-bearing mouse models where focal radiation was administered in combination with TAK-676, with or without an anti-PD-1 antibody. A regimen of 10 Gy × 1 radiation was delivered with the RS 2000 Small Animal Irradiator using a lead box to provide shielding, with a small hole to provide access to the tumor. A regimen of 8 Gy × 3 radiation was delivered with the SARRP (Small Animal Radiation Research Platform) with 3D volumetric image guidance for target localization and dose delivery, which minimizes exposure to nontargeted tissues and organs and allows higher total doses of radiation to be delivered to the tumor.

- Tolerability of TAK-676 at 1 mg/kg in combination with a single fraction of 10 Gy was assessed in 2 pharmacology studies in the EMT6 mouse breast cancer model and in 1 study in the A20 mouse lymphoma model.
  - In 1 study, TAK-676 at 1 mg/kg was administered to EMT6 tumor-bearing mice on a Q3D×3 schedule (Days 2, 5, and 8) alone or in combination with 10 Gy radiation on 1 of 5 different days (Day 0, 1, 2, 3, or 4). A radiation alone arm, where 10 Gy radiation was given once on Day 2 was also included. All groups contained 10 mice each. Transient BWL was observed in all groups. Maximum mean BWL in single agent groups was 4.1% and 2.3% for TAK-676 and radiation respectively, while maximum mean BWL in the 5 combination groups ranged from 7.0% to 10.1%. BWL resulted in the removal of 2 of 50 mice receiving TAK-676 in combination with 10 Gy radiation. These mice, which received TAK-676 + 10 Gy radiation on Day 4, were removed from the study on Days 11 and 33.
  - In another study, TAK-676 at 1 mg/kg was administered to EMT6 tumor-bearing mice Q3D×3 (Days 2, 5, and 8) alone or in combination with 10 Gy radiation on 1 of 3 different days (Day 0, 2, or 4). Radiation alone arms, where 10 Gy radiation was given once on Day 0, 2, or 4 were also included. All groups contained 10 mice each. Transient BWL was observed in all treatment groups but was considered tolerable as mean BWL was <11% in all groups and no mice met criteria for removal from study due to BWL. Maximum mean BWL in single agent groups was 1.7% and 0.9% to 5.2% for TAK-676 and radiation groups respectively, while maximum mean BWL in the 3 combination groups ranged from 9.4% to 11%.

  - In the A20 mouse lymphoma model, TAK-676 at 1 mg/kg was administered to mice Q3D×3 (Days 2, 5, and 8) alone or in combination with 10 Gy radiation on 1 of 3 different days (Day 0, 2, or 4). Radiation alone arms, where 10 Gy radiation was given once on Day 0, 2, or 4 were also included. Transient BWL was observed in all groups which received TAK-676 or radiation as single agents or in combination. Maximum mean BWL in single agent groups was 4.9%, 8%, 9.7%, and 9.3% for TAK-676, Day 0 radiation, Day 2 radiation, and Day 4 radiation respectively. BWL in these single-agent groups resulted in the removal of 1 mouse (of 10) on Day 11 that received 10 Gy radiation on Day 4. In combination groups, BWL was additive, resulting in a maximum mean BWL of 14.3%, 13.6%, and 15.6% for TAK-676 + radiation on Day 0, 2, or 4, respectively. In combination groups, BWL resulted in the early removal of 5 of 10 mice (on Days 4 and 7) with TAK-676 + 10 Gy radiation administered on Day 0, 1 of 10 mice (on Day 9) with TAK-676 + 10 Gy radiation administered on Day 2, and 3 of 10 mice (on Days 7 and 9) with TAK-676 + 10 Gy radiation administered on Day 4.

- Tolerability of TAK-676 in combination with 8 Gy × 3 with or without anti-PD-1 antibody treatment was assessed in a study in EMT6 tumor-bearing mice. In this study, radiation was administered once daily for 3 consecutive days, followed 48 hours later by TAK-676 dosed at 0.25 or 1 mg/kg on a Q3D×3 schedule, along with an anti-PD-1 antibody dosed Q3D×3 on the same days as TAK-676. The regimen of 8 Gy × 3 followed 48 hours later by TAK-676 at 1 mg/kg was also evaluated with an isotype control antibody instead of an anti-PD-1 antibody. For comparison, the study included groups to test single agent effects of 8 Gy × 3 radiation, anti-PD-1 antibody, and TAK-676 at 0.25 mg/kg and 1 mg/kg, and a group which received 8 Gy × 3 radiation followed by anti-PD-1 antibody. All groups contained 8 mice each. Transient BWL was observed in all treatment groups. Maximum mean BWL in single-agent groups was 3.7%, 1.6%, 7.8%, and 0.2% for radiation, 0.25 mg/kg TAK-676, 1 mg/kg TAK-676, and anti-PD-1 groups, respectively. Maximum mean BWL in the group which received radiation and anti-PD-1 was 3.8%. In the groups that received 8 Gy × 3 followed by TAK-676 at 1 mg/kg, with anti-PD-1 or isotype control, the maximum mean BWL was 9.0% and 9.9%, respectively. Maximum mean BWL of the group receiving 8 Gy × 3 with 0.25 mg/kg TAK-676 and anti-PD-1 was 1.8%. The combination was considered tolerable as no mice were removed for BWL during the study.

Taken together, these results indicate that in EMT6 tumor-bearing mice, TAK-676 at 1 mg/kg was well tolerated in combination with 8 Gy × 3 radiation delivered through an advanced image-guided system to minimize normal tissue radiation exposure. BWL was <10% and no treatment-related mortality was observed. The addition of an anti-PD-1 antibody to this regimen did not result in additional BWL or mortality. In EMT6 mice, TAK-676 at 1 mg/kg was also generally well tolerated in combination with 10 Gy × 1 radiation delivered without image guidance, with 7% to 11% maximal mean BWL in 8 combination groups (n = 80 mice total), with the exception of the removal of 2 mice due to BWL. In contrast, A20 tumor-bearing mice treated with TAK-676 at 1 mg/kg and 10 Gy × 1 radiation did not tolerate the regimen, experiencing more BWL than seen in the EMT6 breast cancer model and removal of 9/30 mice during the study. This is likely due to the

greater sensitivity of A20 lymphoma tumor-bearing mice to radiation as a single agent and the additive effect of TAK-676 on BWL.

5.0 STUDY OBJECTIVES AND ENDPOINTS

5.1 Objectives

5.1.1 Primary Objective

The primary objective is:

- To determine the safety and tolerability of TAK-676 administered in combination with pembrolizumab following radiation therapy in patients with locally advanced or metastatic NSCLC, TNBC, or SCCHN.

5.1.2 Secondary Objectives

The secondary objectives are:

- To determine the RP2D of TAK-676 administered in combination with pembrolizumab following radiation therapy. RP2D can be equal to or lower than the MTD.
- To assess the preliminary antitumor activity of TAK-676 administered in combination with pembrolizumab following radiation therapy, both locally (in the radiation field) and systemically (nonirradiated lesions).
- To evaluate the dose-responsive impact on T-cell infiltration in nonirradiated tumors following TAK-676 administered in combination with pembrolizumab following radiation therapy.

5.1.3 Exploratory Objectives

The exploratory objectives are:

- To determine whether TAK-676 administered in combination with pembrolizumab following radiation therapy results in changes in peripheral blood consistent with activation of the innate and/or adaptive immune response.
- To characterize mutations or polymorphisms associated with response or resistance to the combination of TAK-676 and pembrolizumab following radiation therapy, for example, polymorphisms in the STING gene (TMEM173) and drug transporter genes relevant to TAK-676, and in immune response or DNA damage repair genes.
- To characterize plasma concentration of TAK-676 and explore exposure-response relationship.

5.2 Endpoints

5.2.1 Primary Endpoints

The primary endpoints are:

- Frequency and severity of treatment-emergent adverse events (TEAEs).
- Number of patients with DLTs.
- Number/percentage of patients with 1 or more treatment-emergent serious adverse event (TESAE).
- Number/percentage of patients with 1 or more TEAE leading to dose modifications and treatment discontinuation.

Safety endpoints will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 5.0.

5.2.2 Secondary Endpoints

The secondary endpoints are:

Response assessments are to be made by the investigator per Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 (Eisenhauer et al. 2009) and also per a modified intratumoral immunotherapy Response Evaluation Criteria in Solid Tumors (itRECIST) (where intratumoral therapy references are largely replaced with radiation therapy). To allow for instances of pseudoprogression, allowances will be made for patients to continue on treatment after an initial assessment of PD, assuming subsequent imaging does not confirm PD. See section 9.4.14.1 for details regarding this allowance.

Response assessments are per RECIST v 1.1 by investigator

- ORR: confirmed complete response (cCR) + confirmed partial response (cPR).
- Duration of response (DOR) for all tumor lesions assessed by RECIST v.1.1
- Time to response (TTR) for all tumor lesions assessed by RECIST v.1.1

Response assessments are per modified itRECIST by investigator

- Overall response rate (ORR): cCR + cPR.
- Overall response rate for tumors lying within the radiation field (ORRirradiated): confirmed complete response [cCRirradiated] + confirmed partial response [cPRirradiated] of tumor lesions lying within the radiation field.
- Overall response rate for tumors lying outside the radiation field (ORRnonirradiated): confirmed complete response [cCRnonirradiated] + confirmed partial response [cPRnonirradiated] of tumor lesions lying outside of the radiation field.

- DOR for tumors lying within the radiation field (DORirradiated), and for those lying outside of the radiation field (DORnonirradiated).
- TTR for tumors lying within the radiation field (TTRirradiated), and for those lying outside of the radiation field (TTRnonirradiated).

The following endpoint will be assessed to evaluate T-cell infiltration into the tumor between pretreatment and on-treatment biopsies:

- Cell infiltration evaluated by immunohistochemistry.

5.2.3 Exploratory Endpoints

The exploratory endpoints are:

- Changes in levels of plasma biomarkers, including cytokines and chemokines.
- Changes between pretreatment and on-treatment peripheral blood samples in gene expression, including the STING agonism/type I IFN signature.
- Plasma concentrations of TAK-676.
- Relationship between response and polymorphisms in the STING gene (*TMEM173*) or drug transporter genes relevant to TAK-676.
- Relationship between response and mutations or polymorphisms in immune response or DNA damage repair genes.

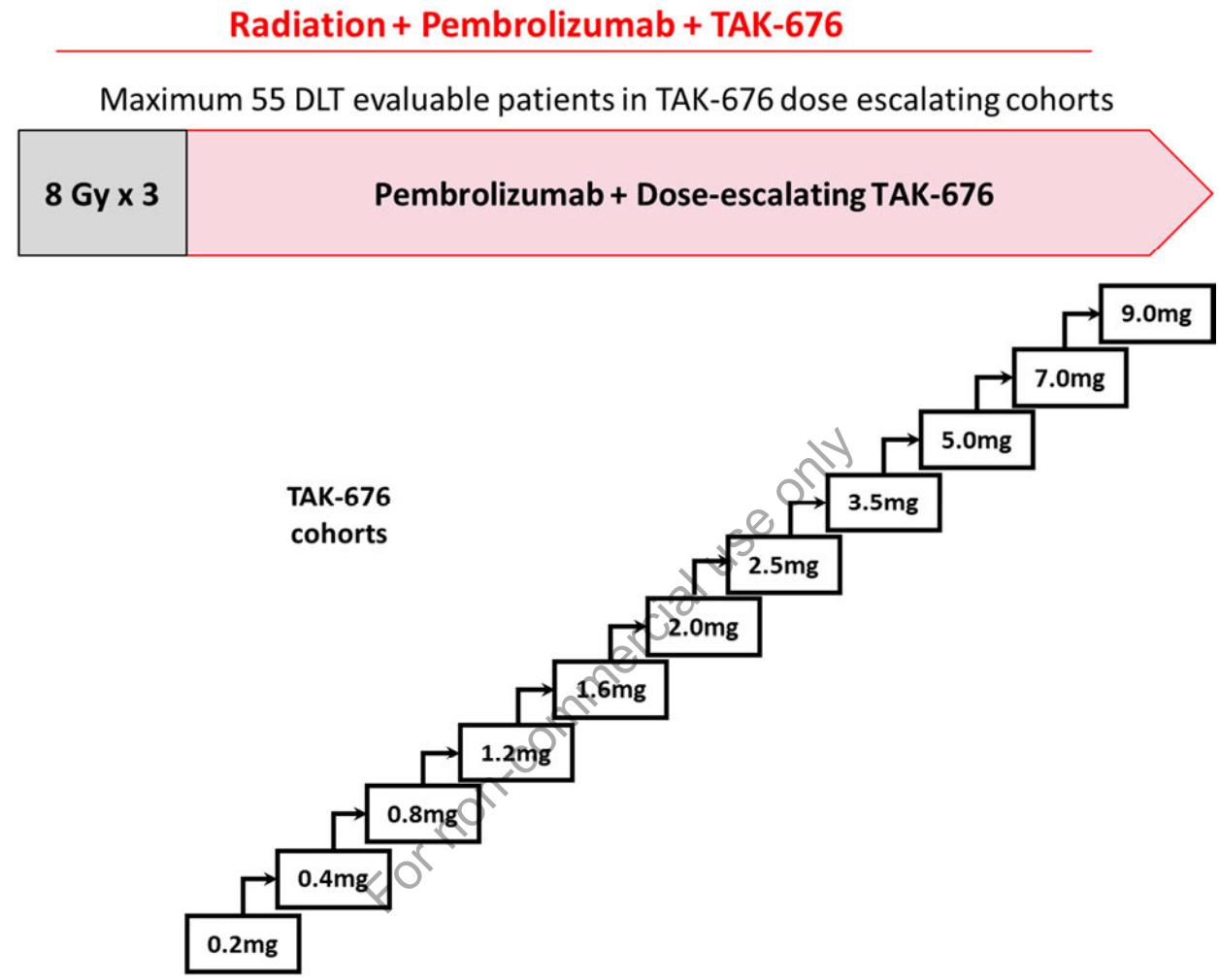
6.0 STUDY DESIGN

6.1 Overview of Study Design

This is an open-label, phase 1, dose escalation study to evaluate the safety, tolerability and preliminary antitumor activity of TAK-676 and pembrolizumab following radiation therapy in the treatment of NSCLC, TNBC, or SCCHN patients who have progressed on CPIs. The information obtained during this study will be used to estimate the MTD and determine the RP2D of this combination.

The study design is depicted in Figure 6.a.

Figure 6.a Study Design



Approximately 65 patients with metastatic NSCLC, TNBC, or SCCHN will be enrolled in this study to achieve a maximum of 55 DLT evaluable patients in TAK-676 dose escalating cohorts. They will receive 8 Gy × 3 radiation therapy, followed by IV administration of pembrolizumab and TAK-676. Pembrolizumab will be administered at 200 mg IV every 3 weeks, with a minimum for 40 hours between the last radiation therapy fraction and the initiation of IV pembrolizumab + TAK-676. Three patients will be enrolled in the initial cohort at the previously identified starting dose level of TAK-676. Subsequent cohorts may enroll 2 to 3 patients per the escalation/de-escalation guidelines outlined in Table 6.a. It is generally expected that at least 3 patients will enroll per cohort. However, if no DLTs have been identified in the TAK-676 + pembrolizumab dose level that has already been evaluated in the dose-finding phase 1 TAK-676-1002 study, the sponsor, in agreement with the TAK-676-1003 investigators, may opt to enroll a 2-patient cohort at that same [radiation] + TAK-676 + pembrolizumab dose level being evaluated in the TAK-676-1003 study. Administration of pembrolizumab will continue every 3

weeks until disease progression, intolerance to pembrolizumab (defined as the development of a TEAE that is at least possibly related to pembrolizumab and for which dose discontinuation is recommended), or withdrawal of consent, whichever occurs first. TAK-676 will be administered in a dose-escalating fashion following the Bayesian Optimal INterval (BOIN) design (Liu and Yuan 2015), with an explorable dose range of 0.2 to 9.0 mg administered on Days 1, 8, and 15 of every 21-day cycle.

Patients will receive TAK-676 with pembrolizumab at dose levels that were previously deemed safe in the dose-finding phase 1 study TAK-676-1002.

AEs will be assessed, and laboratory values, vital signs, ECGs, and other clinically indicated examinations will be obtained to evaluate the safety and tolerability of the study drugs in combination with radiation. Toxicity will be evaluated according to NCI CTCAE, Version 5.0. DLTs are defined in Section 8.2. A DLT will be defined as any of the treatment-emergent AEs (TEAEs) described in the safety evaluation section that occur during Cycle 1 and are considered by the investigator to be at least possibly related to TAK-676 in combination with pembrolizumab and radiation. TEAEs meeting DLT definitions occurring in later cycles will be considered in the determination of RP2D of TAK-676.

Radiological evaluations (CT scan and/or magnetic resonance imaging [MRI] as clinically indicated) will be employed to assess the status of the patient's underlying disease. Before radiation therapy, the radiology oncologist and medical oncologist should agree on the target lesions identified for irradiation using the baseline imaging assessments. Banked formalin fixed paraffin-embedded tumor tissue or a minimum number of unstained slides of the tumor tissue will be collected, if available, from all enrolled patients to assess baseline features such as gene mutations, gene signatures, tumor mutation burden, immune cell content, or biomarkers of response or resistance to treatment that may emerge from future nonclinical or clinical studies. All patients with a safely accessible lesion outside the radiation field and in whom a fresh tumor biopsy enrolling at dose levels of TAK-676 which have been shown to have pharmacodynamic activity will have mandatory tumor biopsy performed as per Schedule of Events (SOE) (Appendix A).

Serial blood samples will be collected for circulating biomarkers (peripheral proteins, cytokines, and chemokines, including IP-10, an IFN-inducible chemokine), immunophenotyping, mRNA expression, receptor sequencing, and cell-free DNA. An evaluation of disease response will be performed using the RECIST v.1.1 (as determined by the investigator) and as per SOE (Appendix A). Serial blood samples for determination of the plasma concentration of TAK-676 and related metabolites to understand TAK-676 metabolism and excretion mechanisms will be obtained at prespecified time points as described in the SOE (Appendix A).

6.1.1 Dose Escalation of TAK-676

Dose escalation of TAK-676 will follow BOIN design to inform dose escalation decisions and potential MTD estimation (Appendix E). Three patients will be enrolled in the initial cohort at the previously identified starting dose level of TAK-676. Subsequent cohorts will enroll 2 to 3 patients per the escalation/de-escalation guidelines outlined in Table 6.a. The target toxicity rate is $\phi = 0.3$.

It is generally expected that at least 3 patients will enroll per cohort. However, if no DLTs have been identified in the TAK-676 + pembrolizumab dose level that has already been evaluated in the dose-finding phase 1 TAK-676-1002 study, the sponsor, in agreement with the TAK-676-1003 investigators, may opt to enroll a 2-patient cohort at that same [radiation] + TAK-676 + pembrolizumab dose level being evaluated in the TAK-676-1003 study. To guide dose escalation decisions, if the observed DLT rate at the current dose is ≤0.236, the next cohort of patients will be treated at the next higher dose level; if it is ≥0.358, the next cohort of patients will be treated at the next lower dose level; if it is within 0.236 and 0.358, additional patients will be enrolled in this dose level. For the purpose of overdose control, dose j and higher levels will be eliminated from further examination if $Pr(p_j>0.3 \mid data) >0.95$, where p_j is the true DLT rate of dose level j. When the lowest dose is eliminated, dose escalation will be stopped for safety. The trial design is illustrated in Table 6.a. Dose escalation will continue until the maximum sample size (55 DLT-evaluable patients) is reached or the number of DLT-evaluable patients treated at the current dose reaches 9. Isotonic regression method will be used on the cumulative DLT rate for each dose level to determine the MTD, defined as the highest TAK-676 dose in combination with radiation therapy and pembrolizumab that does not result in unacceptable toxicities.

Table 6.a Dose Escalation/De-escalation Rule for the BOIN Design for 55 DLT-Evaluable Patients

Number of Patients Treated at the Current Dose	1	2	3	4	5	6	7	8	9
Escalate if number of DLT ≤	0	0	0	0	1	1	1	1	2
De-escalate if number of DLT ≥	1	1	2	2	2	3	3	3	4
Eliminate if number of DLT ≥	NA	NA	3	3	4	4	5	5	5

BOIN: Bayesian Optimal Interval; DLT: dose-limiting toxicity; NA: not applicable.
Number of DLTs is the number of patients with at least 1 DLT.

Patients not receiving all required doses of radiation, TAK-676, and pembrolizumab through Cycle 1 for reasons other than DLTs will not be considered DLT evaluable but may remain on study. If a patient is DLT inevaluable, patient replacement may not be mandatory except for the first cohort. In this case, the decision for patient replacement can be determined based on the number of DLT-evaluable patients in the cohort, as agreed between the sponsor and investigators.

Other dose escalation decisions, evaluation of intermediate doses, expansion of an existing dose level, and stopping the dose escalation early are all permissible following discussions between the sponsor and the investigators, if such measures are needed for patient safety, or for a better understanding of the dose-related toxicity, exposure, and/or pharmacodynamics. Other non-DLT safety and available clinical, PK, or biomarker data will also be considered to inform subsequent dose recommendations, dose escalation decisions, and potential MTD estimation.

6.2 Number of Patients

Approximately 65 patients with metastatic NSCLC, TNBC, or SCCHN will be enrolled in this study, to achieve a maximum of 55 DLT evaluable patients for the dose escalation of TAK-676 administered along with pembrolizumab following radiation therapy.

6.3 Duration of Study

6.3.1 Duration of an Individual Patient's Study Participation

Patient participation will include screening, treatment, and follow-up. Screening will last up to 28 days before the first dose of radiation, during which the patient's eligibility and baseline characteristics will be determined. Treatment with TAK-676 with pembrolizumab will be administered for up to 24 months or until patients meet any of the discontinuation criteria in Section 8.4.5, Section 8.4.6, or Section 9.7. Patients with demonstrated clinical benefit may continue treatment beyond 24 months with the agreement of the sponsor. These patients can continue receiving treatment in this study or any of the poststudy access modalities described in Section 6.3.5.

All patients will attend an end of treatment (EOT) visit 30 days (+10 days) after receiving their last dose of study drug or before the start of subsequent systemic anticancer therapy, whichever occurs first, to permit detection of any delayed TEAEs and to resolve any ongoing events. If a patient is not able to return for the EOT visit, the EOT assessments may be performed at the time of treatment discontinuation following discussion with the sponsor. Patients with unresolved TEAEs will continue the periodic safety follow-up until complete resolution or stabilization (established as sequelae) occurs. Patients who receive at least 1 dose of radiation and discontinue study treatment for reasons other than PD will continue follow-up every 12 ±1 weeks from the EOT visit until the occurrence of PD, loss to follow-up, consent withdrawal, the start of subsequent systemic antineoplastic therapy, study termination, or death, whichever occurs first (Section 9.8).

6.3.2 End of Study/Study Completion Definition and Planned Reporting

The anticipated duration of this study will be approximately 30 months (from first patient first visit to last patient last visit). The final data cutoff for the CSR will be conducted after all patients have been discontinued from treatment or transferred to a long-term safety study or a similar program (Section 6.3.5).

Patients previously discontinued from study treatment will undergo the EOT visit but will continue to be followed every 12 ±1 weeks from the EOT visit as described in Section 9.8.

6.3.3 Timeframes for Primary and Secondary Endpoints to Support Disclosures

Refer to Table 6.b for disclosures information for all primary and secondary endpoints.

Table 6.b Primary and Secondary Endpoints for Disclosures

Endpoint	Definition	Maximum Time Frame [a]
Primary:		
Frequency and severity of TEAEs	Standard safety assessments	Up to ~30 months
Number of patients with DLTs	Standard safety assessments	Up to ~30 months
Number/percentage of patients with 1 or more treatment-emergent SAE	Standard safety assessments	Up to ~30 months
Number/percentage of patients with 1 or more TEAE leading to dose modifications and treatment discontinuations	Standard safety assessments	Up to ~30 months
Secondary [b]:		
ORR	ORR	Up to ~30 months
ORRnonirradiated	ORRnonirradiated	Up to ~30 months
ORRirradiated	ORRirradiated	Up to ~30 months
DOR	DOR	Up to ~30 months
DORnonirradiated	DORnonirradiated	Up to ~30 months
DORirradiated	DORirradiated	Up to ~30 months
TTR	TTR	Up to ~30 months
TTRnonirradiated	TTRnonirradiated	Up to ~30 months
TTRirradiated	TTRirradiated	Up to ~30 months
T-Cell infiltration into the tumor between pretreatment and on-treatment biopsies	Standard pharmacodynamic assessment	Up to ~30 months

DLT: dose-limiting toxicity; DOR: duration of response; itRECIST: modified intratumoral immunotherapy Response Evaluation Criteria in Solid Tumors; RECIST: Response Evaluation Criteria in Solid Tumor; ORR: overall response rate; ORRirradiated: overall response rate for tumors lying within the radiation field; ORRnonirradiated: overall response rate for tumors lying outside of the radiation field; SAE: serious adverse event; TEAE: treatment-emergent adverse event; TTR: time to response; TTRirradiated: time to response for tumors lying within the radiation field; TTRnonirradiated: time to response for tumors lying outside of the radiation field.

[a] Maximum time frame to last assessment for that endpoint for the study.

[b] Assessments are per RECIST v.1.1 and itRECIST.

6.3.4 Total Study Duration

It is anticipated duration of this study will be approximately 30 months (from first patient first visit to last patient last visit).

6.3.5 Post-trial Access

Patients who are still on study after the estimated study completion time of approximately 30 months will be allowed to continue pembrolizumab with or without TAK-676 in an extension phase of this study, in a separate open-label rollover study, or through a single-patient Investigational New Drug application or equivalent. Patients who discontinue TAK-676 for reasons other than disease progression will continue to receive pembrolizumab as long as clinical

benefit is documented. The mechanism of access will depend on the number of patients who require it. This access will be permitted only when the investigator and sponsor confirm that a patient has experienced a clinically important response to the combination that outweighs the potential risks of continued treatment. Additionally, these patients should have no comparable or satisfactory alternative therapeutic option and would be negatively affected without continued access. If pembrolizumab is commercially available and reimbursable for a given indication, pembrolizumab can be reimbursed by Takeda (if Takeda reimbursed the site while the patient was on study) for up to 12 months after study completion, at which time it should then be sourced commercially for continued administration with or without TAK-676 if the patient is still benefiting. If there are any issues in obtaining authorization for use of pembrolizumab, these should be discussed with Takeda and handled on a case by case basis. If pembrolizumab is not commercially available and reimbursable in a given indication, it will continue to be reimbursed by Takeda for continued administration with or without TAK-676 until any of the conditions in Section 6.3.5.1 are met.

6.3.5.1 Duration of Post-trial Access

Continued access to pembrolizumab with or without TAK-676 will be terminated for those individuals who no longer benefit (eg, they have completed the recommended course of therapy, or their disease has progressed), the benefit-risk no longer favors the individual, if pembrolizumab with or without TAK-676 becomes available either commercially (see preceding paragraph) or via another access mechanism, or when an alternative appropriate therapy becomes available. Poststudy access may be terminated in a country or geographical region where marketing authorization has been rejected, the development of TAK-676 has been suspended or stopped by the sponsor, or TAK-676 in combination with pembrolizumab can no longer be supplied.

7.0 STUDY POPULATION

7.1 Inclusion Criteria

Each patient must meet all the following inclusion criteria to be enrolled in the study:

1. Adult male or female patients, aged 18 years or older.
2. Voluntary written consent must be given before performance of any study-related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.
3. Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1.
4. Patients must have at least 2 measurable lesions (ie >10 mm longest diameter for extranodal lesions, >15 mm short axis for lymph nodes), with at least one inside and at least one other outside of the radiation field. The tumor outside the radiation field must be accessible for biopsy, and the patient must consent to tumor biopsy at screening and during treatment as described in the SOE (Appendix A).

5. Patients with pathologically confirmed (cytological diagnosis is adequate) advanced or metastatic NSCLC, TNBC, or SCCHN who have:
   a) Received or been offered all established SOC treatment options for which they are eligible; and
   b) Progressed on CPIs in a prior line of therapy.
6. Not Applicable. In Protocol Amendment 2, requirement of patient having life expectancy >12 weeks has been removed.
7. Adequate bone marrow, renal, and hepatic functions, as determined by the following laboratory parameters:
   a) Absolute neutrophil count (ANC) ≥1000/μL, platelet count ≥75,000/μL, and hemoglobin ≥8.0 g/dL without growth factor support for neutrophils or transfusion support for platelets within 14 days before the first study treatment dose.
   b) Total bilirubin ≤1.5 times the institutional ULN. For patients with Gilbert's disease, ≤3 mg/dL.
   c) Serum ALT and AST ≤3.0 times the ULN (or ≤5.0 times the ULN with presence of liver metastases).
   d) Albumin ≥3.0 g/dL.
   e) Estimated creatinine clearance using the Cockcroft-Gault formula ≥30 mL/min.
8. Left ventricular ejection fraction (LVEF) >50%, as measured by echocardiogram or multiple-gated acquisition (MUGA) scan within 4 weeks before receiving the first dose of study drug.
9. Clinically significant toxic effects of previous therapy have recovered to Grade 1 (per NCI CTCAE, Version 5.0) or baseline, except for alopecia, Grade 2 peripheral neuropathy, and/or autoimmune endocrinopathies with stable endocrine replacement therapy.
10. Female patients must be:
    a) Postmenopausal (natural amenorrhea and not due to other medical reasons) for at least 1 year before the screening visit, OR
    b) Surgically sterile, OR
    c) If they are of childbearing potential, agreeable to practicing 2 effective methods of contraception at the same time, from the time of signing the informed consent through 120 days after the last dose of study drug(s), OR
    d) Agreeable to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient.

    Note: Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods), withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.

11. Male patients, even if surgically sterilized (ie, status postvasectomy), must:
    a) Agree to practice effective barrier contraception during the entire study treatment period and through 120 days after the last dose of study drug, OR
    b) Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient.

    Note: Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods), withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.

7.2 Exclusion Criteria

Patients having any of the following exclusion criteria are not to be enrolled in the study:

1. History of any of the following ≤6 months before first dose of study drug(s): congestive heart failure New York Heart Association Grade III or IV (Appendix G), unstable angina, myocardial infarction, persistent hypertension ≥160/100 mm Hg despite optimal medical therapy, ongoing cardiac arrhythmias of Grade >2 (including atrial flutter/fibrillation or intermittent ventricular tachycardia), other ongoing serious cardiac conditions (eg, Grade 3 pericardial effusion or Grade 3 restrictive cardiomyopathy), or symptomatic cerebrovascular events. Chronic, stable atrial fibrillation on stable anticoagulation therapy, including low molecular-weight heparin, is allowed.
2. History of brain metastasis unless:
    a) Clinically stable, (ie, treatment completed ≥4 weeks prior) following prior surgery, whole-brain radiation, or stereotactic radiosurgery, AND
    b) Off corticosteroids.
3. Known history of uncontrolled autoimmune disorders, HIV infection, or other relevant congenital or acquired immunodeficiencies.
4. Chronic, active hepatitis (eg, patients with known hepatitis B surface antigen seropositive and/or detectable hepatitis C virus [HCV]-RNA).

    Note: Patients who have positive hepatitis B core antibody can be enrolled but must have an undetectable serum hepatitis B virus-DNA. Patients who have positive HCV antibody must have an undetectable HCV-RNA serum level.
5. Contraindication and/or history of intolerance to the administration of CPI.
6. Contraindication and/or history of intolerance to the radiation therapy.
7. Any illness, metabolic dysfunction, physical examination finding, or clinical laboratory finding that give reasonable suspicion of a disease or condition that would contraindicate the use of an investigational drug or that would limit compliance with study requirements or compromise ability to provide written informed consent.

8. Treatment with any investigational products and systemic anticancer drugs (including vascular endothelial growth factor inhibitors), within 14 days or 5 half-lives, whichever is shorter, before Cycle 1 Day 1 (C1D1) of study drug(s).
9. Concurrent chemotherapy, immunotherapy (except for pembrolizumab), biologic, or hormonal therapy (except for adjuvant endocrine therapy for a history of breast cancer). Concurrent use of hormones for noncancer-related conditions is acceptable (eg, corticosteroid replacement use is outlined in Section 8.5).
10. Prior radiation to lesions chosen for biopsy or response assessment.
11. Prior radiation to lesions other than those chosen for radiation therapy or biopsy in the current protocol within 4 weeks of C1D1 of study drug(s).
12. Use of systemic corticosteroids or other immunosuppressive therapy, concurrently or within 7 days of start of radiation therapy, with the following exceptions:
    a) Topical, intranasal, inhaled, ocular, intra-articular, and/or other nonsystemic corticosteroids.
    b) Physiological doses of replacement steroid therapy (eg, for adrenal insufficiency).
13. Receipt of live attenuated vaccine (eg, tuberculosis Bacillus Calmette-Guerin [BCG] vaccine, oral polio vaccine, measles, rotavirus, yellow fever) within 28 days of C1D1 of study drug(s).
14. Recipients of allogeneic or autologous stem cell transplantation or organ transplantation.
15. Female patients who are lactating or have a positive serum pregnancy test during the screening period or a positive urine pregnancy test on Day 1 before first dose of study drug.

    Note: Female patients who are lactating will be eligible if they choose to discontinue breastfeeding before the first dose of study drug.
16. Ongoing Grade ≥2 infection or patients with Grade ≥2 fever of malignant origin.
17. Fridericia's corrected QT interval (QTcF) >450 milliseconds (msec) (males) or >475 msec (females) on a 12-lead ECG during the screening period.
18. Grade ≥2 hypotension (ie, hypotension for which nonurgent intervention is required) at screening or during C1D1 predose assessment.
19. Oxygen saturation <92% on room air at screening or during C1D1 predose assessment.
20. Use of medications that are known clinical OATP1B1 and/or OATP1B3 inhibitors, concurrently or within 14 days of C1D1 of study drug(s) (Appendix I).
21. Patients treated with other STING agonists/antagonist and toll-like receptors agonists within the past 6 months.
22. Current smoker.
23. Vaping within 90 days of C1D1 of study drug(s).

24. Current diagnosis of pneumonitis, interstitial lung disease, severe chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, other restrictive lung diseases, acute pulmonary embolism, or Grade ≥2 pleural effusion or ascites not controlled by tap or requiring indwelling catheters.

8.0 STUDY DRUG

All protocol-specific criteria for administration of radiation and study drug(s) must be met and documented before drug administration. Radiation therapy and study drug(s) will be administered under the supervision of the investigator or identified subinvestigator(s).

Investigational medicinal product(s): radiation therapy, pembrolizumab, TAK-676.

8.1 Study Drug Administration

8.1.1 Radiation Treatment Regimen

Before radiation therapy, the radiology oncologist and medical oncologist should agree on the target lesions identified for irradiation using of the baseline imaging assessments.

Radiation therapy with daily image guidance will be administered using a fractionated dose of 8 Gy × 3.

All patients will receive radiation therapy and pembrolizumab in addition to an escalating dose of TAK-676.

The radiation must be administered between Day -8 and Day -2 with a minimum of 40 hours between the last fraction of radiation and initiation of IV pembrolizumab and TAK-676. At least 1 and up to 3 lesions may be radiated, if indicated for palliative reasons. If the initial planned dose of 8 Gy × 3 results in intolerable local AEs when given in combination with TAK-676 and pembrolizumab, the radiation dose may be reduced to 8 Gy × 1 in subsequent cohorts.

The radiation treatment and planning process will proceed as follows:

1. Immobilization.
   a) Patients will be positioned in a stable and comfortable position, allowing accurate reproducibility of the target position from treatment to treatment.
   b) A variety of immobilization systems may be used, including stereotactic frames, patient-customized rigid pillows, and thermoplastic masks.
2. Radiation CT simulation.
   a) The treatment planning process will include CT based simulation with axial imaging every 1 to 3 mm to cover the area(s) of interest.
   b) IV and/or oral contrast during the planning CT is optional.

3. Internal motion management.
   a) Internal tumor and organ motion (eg, due to breathing) must be accounted for during the simulation and planning process.
   b) For targets (eg, lung, liver, adrenal and thoracic/upper abdominal lymph node targets) that have significant motion, 4D-CT planning is mandatory.
   c) Acceptable motion management includes reliable abdominal compression, linear accelerator beam gating with the respiratory cycle, tumor tracking, and active breath-holding techniques.
4. Daily target localization.
   a) Image-guided radiation therapy is mandatory.
   b) Contours: Tumor contours will be defined by the physician and radiation oncology staff:
      i. Gross tumor volume (GTV): This is the target gross tumor as defined by the treating radiation oncologist with the help of positron emission tomography, MRI, and diagnostic CT with contrast as appropriate.
      ii. Internal target volume (ITV): Delineation of an ITV is mandated when a 4D-CT is required.
      iii. Clinical tumor volume (CTV): An optional 0- to 1-cm margin on GTV is allowed when determining the CTVs at the treating radiation oncologist's discretion to account for any uncertainty or microscopic disease. This margin may be expanded based on clinical judgment with permission of the principal investigator.
      iv. Planning tumor volume (PTV): A 3 to 5 mm PTV margin will be uniformly added to the CTV and will also be included to account for set-up variation, as appropriate to each individual lesion.
      v. Organs at risk (OARs): For the 8 Gy × 3 dose level, several contours of the OAR will be defined by the physician and/or treatment planner if visible within the axial slices within 2 cm of the PTV as per institutional guidelines and indicated in Table 8.a or unless noted below:
         - Spinal cord: In cases where the PTV overlaps the vertebral column and the dose fractionation is greater than 8 Gy × 1, the spinal cord will be delineated with the aid of interventional radiology myelogram or fused MRI. Otherwise, the spinal cord will be contoured based on the bony limits of the spinal canal. The spinal cord should be contoured starting at least 5 cm above the superior extent of the PTV and continuing on every CT slice to at least 5 cm below the inferior extent of the PTV.
         - Heart/pericardium: The heart will be contoured along with the pericardial sac. The superior aspect will begin at the level of the inferior aspect of the pulmonary artery passing the midline and extend inferiorly to the apex of the heart.

- Proximal bronchial tree: This structure includes the distal 2 cm of the trachea, the carina, the right and left mainstem bronchi, the right and left upper lobe bronchi, the intermedius bronchus, the right middle lobe bronchus, the lingular bronchus, and the right and left lower lobe bronchi.
- Skin: The skin will be defined as the outer 0.5 cm of the body surface.
- Bowel: Loops of large bowel and small bowel will be contoured separately. Small bowel will be evaluated using the duodenum constraints in TG-101 as they are more conservative.

Table 8.a Target Region and Contours

Target Region	Minimum OARs to contour if on the same axial slice or within 2 cm craniocaudal of PTV
Head and neck	Larynx, spinal cord, brachial plexus, esophagus, lungs, brainstem, skin
Thoracic	Lungs, brachial plexus, trachea, esophagus, proximal bronchial tree, skin, heart, stomach, liver, spinal cord, kidneys, small bowel, large bowel
Abdominopelvic	Lungs, esophagus, skin, heart, stomach, liver, spinal cord, kidneys, small bowel, large bowel, rectum, bladder, femoral heads, sacral plexus, cauda equina

OAR: organ at risk; PTV: planning tumor volume.

Dose constraints as per TG-101 (Appendix J) will be prioritized over target coverage.

The table below provides the biologically equivalent dose of the fractionation scheme based on linear-quadratic model (Brenner 2008) and assuming and α/β of 3.

Table 8.b Biologically Equivalent Dose for the Radiation Fractionation Scheme Based on the Linear-Quadratic Model and Assuming an α/β of 3

Dose Fractionation Scheme	Biologically Effective Dose (BED3)	Biologically Equivalent Dose in 2 Gy Fractions (EQD2)
8 Gy × 3 fractions	88.0 Gy_3	52.8 Gy

Source: (Brenner 2008).
Gy: gray.
This was selected for normal tissue toxicity concerns given this is primarily a safety study.

8.1.2 Pembrolizumab

Route of administration: IV infusion over 30 minutes.

Dose level: 200 mg.

Pembrolizumab will be administered on C1D1 at 200 mg IV following the label instructions at least 40 hours after the last fraction of radiation and on an every 3-week basis thereafter. Administration will continue until disease progression, intolerance to pembrolizumab (defined as

the development of a TEAE that is at least possibly related to pembrolizumab and for which dose discontinuation is recommended), or withdrawal of consent, whichever occurs first.

For administrations of pembrolizumab, vital signs will be measured immediately before the start of the infusion (within 30 minutes) and any time when clinically indicated.

For preparation, handling, and administration instructions related to pembrolizumab, please consult the Pharmacy Manual and/or the product information included with pembrolizumab (eg, package insert).

8.1.3 TAK-676

Route of administration: IV infusion over 60 ±10 minutes.

Dose level: 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.5, 3.5, 5.0, 7.0, and 9.0 mg.

TAK-676 infusion will start 1 hour (+15 minutes) after the end of pembrolizumab infusion on Day 1 of each cycle.

TAK-676 will be provided as a solution containing 3 mg/3 mL TAK-676 drug substance as free base. TAK-676 injection 3 mg/3 mL as free base solution needs to be diluted for infusion at the clinical site (refer to the Pharmacy Manual).

TAK-676 will be administered IV on a dosing schedule of Days 1, 8, and 15 of a 21-day cycle. Alternate dosing schedules of administration in combination with pembrolizumab may also be considered during dose escalation if the collective data including safety, PK, and pharmacodynamics support it, without requiring a protocol amendment.

Administration should always be performed on schedule; however, if extenuating circumstances prevent a patient from beginning treatment on a particular dosing day, a -1/+2-day window will be allowable for all cycles except for Cycle 1. During subsequent cycles, if the Day 1 dose schedule is altered, it is preferred to also shift the Day 8 and Day 15 doses and maintain a 21-day cycle. If the Day 8 or Day 15 dose scheduled is altered, it is preferred to return to the original schedule for the subsequent dose. If a patient misses a dose outside of this window, that dose will be considered missed and dosing will resume at the next scheduled dosing timepoint. Based on emerging data (eg, severity of infusion-related reactions), infusion time may be adjusted to less than or greater than 60 minutes upon agreement with the sponsor and investigators. The infusion may be slowed or stopped and restarted for any infusion-related reactions. All infusion times must be recorded. Premedication for infusion is not recommended initially, but also may be added based on emerging data and upon agreement with the sponsor and investigators.

For administrations of TAK-676, vital signs will be measured immediately before the start of the infusion (within 30 minutes), 30 minutes after start of infusion (±5 minutes), at the end of infusion (±10 minutes), 2 hours post infusion (±30 minutes), any time when clinically indicated, and before discharge (if different from and not overlapping with the timepoints above). Vital signs should be measured and recorded at any time if the patient develops symptoms associated with potential risks of TAK-676 and/or pembrolizumab. The frequency and timing of vital sign monitoring may be modified based on emerging data and agreement between sponsor and investigators.

TAK-676 administration will occur in facilities with readily available resuscitation equipment, diagnostic equipment and supportive care/medications such as oxygen, antihistamines, acetaminophen, corticosteroids, epinephrine, tocilizumab or other anti-IL-6 agents, and bronchodilators.

As with other potentially toxic compounds, caution should be exercised in handling this drug. The use of gloves is recommended. Given the possibility of extravasation, it is advisable to closely monitor the infusion site per nursing institutional guidelines for possible infiltration during drug administration. Administration through a central port is always preferred vs a peripheral line.

For detailed information on the preparation and administration of TAK-676, refer to the Pharmacy Manual.

8.2 Definitions of DLT

A DLT will be defined as any of the following TEAEs that occur during Cycle 1 and is considered by the investigator to be at least possibly related to TAK-676 in combination with pembrolizumab (note that AEs in which the relationship to study drug[s] cannot be ruled out should be considered possibly related to study drug[s]):

1. Any Grade 5 TEAE.
2. Grade 4 anemia.
3. Grade ≥4 neutropenia lasting ≥7 days or requiring use of granulocyte colony stimulating factor (G-CSF).
4. Any febrile neutropenia.
5. Platelet count <10,000/μL at any time.
6. Grade 4 thrombocytopenia lasting ≥7 days, or Grade ≥3 thrombocytopenia associated with clinically significant bleeding.
7. Grade ≥3 CRS.
8. Grade ≥2 immune-mediated uveitis that does not respond to topical therapy and does not improve to Grade ≤1 severity within 2 weeks of the initiation of topical therapy OR requires systemic treatment.
9. Delay in the initiation of Cycle 2 by more than 21 days from the calculated start date due to a lack of adequate recovery of treatment-related hematological or nonhematologic toxicities.
10. Development of Grade ≥3 myelitis, pneumonitis, gastritis, hepatitis, dermatitis or pain flare in the relevant radiation field.
11. Any Grade ≥3 nonhematologic toxicity with the following exceptions:
    a) Grade 3 arthralgia/myalgia that responds to nonsteroidal anti-inflammatory drugs within 1 week.
    b) Grade 3 fatigue lasting <7 days.

c) Any Grade 3 endocrinopathy that is adequately controlled by hormonal replacement.

d) Grade 3 or 4 inflammatory reaction attributed to a local antitumor response (defined as local pain, irritation, or rash localized at sites of known or suspected tumor).

e) Transient (≤24 hours) Grade 3 flu-like symptoms that resolve spontaneously or are controlled with medical management.

f) Grade 3 or 4 asymptomatic laboratory changes (other than renal and hepatic laboratory values) that can be successfully corrected (reversion of Grade ≤1 or baseline) within 72 hours.

g) Isolated elevation of ALT and/or AST, that is, ≤10 times the ULN in the absence of significant bilirubin elevation (Grade <3), excluding elevations meeting Hy's Law.

h) Grade 3 nausea and/or emesis that can be controlled to Grade ≤1 in ≤3 days with the use of antiemetics (such as metoclopramide, prochlorperazine, 5-hydroxytryptamine [serotonin] type 3 receptor antagonist, and/or neurokinin-1 receptor antagonists).

i) Grade 3 rash and pruritis that respond to a standard treatment and resolve or improve to Grade <3 within 7 days.

j) Grade 3 diarrhea that can be controlled to Grade ≤2 in ≤3 days with supportive treatment.

k) Alopecia.

To the extent possible, administration of coronavirus disease 2019 (COVID-19) vaccinations should be avoided during the Cycle 1 DLT window; however, vaccination timing remains at the discretion of the investigator following the guidance in Section 8.5.

TEAEs meeting DLT definitions occurring in Cycle 2 or later will be considered in the determination of the RP2D of TAK-676 (that might be equal to or lower than the MTD).

8.3 Dose Escalation Rules

Dose escalation of TAK-676 will follow BOIN design to inform dose escalation decisions and potential MTD estimation. Three patients will be enrolled in the initial cohort at the previously identified starting dose level of TAK-676. Subsequent cohorts will enroll 2 to 3 patients per the escalation/de-escalation guidelines outlined in Table 6.a. The target toxicity rate is $\phi = 0.3$. It is generally expected that at least 3 patients will enroll per cohort. However, if no DLTs have been identified in the TAK-676 + pembrolizumab dose level that has already been evaluated in the dose-finding phase 1 TAK-676-1002 study, the sponsor, in agreement with the TAK-676-1003 investigators, may opt to enroll a 2-patient cohort at that same [radiation] + TAK-676 + pembrolizumab dose level being evaluated in the TAK-676-1003 study. To guide dose escalation decisions, if the observed DLT rate at the current dose is ≤0.236, the next cohort of patients will be treated at the next higher dose level; if it is ≥ 0.358, the next cohort of patients will be treated at the next lower dose level; if it is within 0.236 and 0.358, additional patients will be enrolled in this dose level. For the purpose of overdose control, dose j and higher levels will be eliminated from further examination if $Pr(p_j>0.3 \mid data) >0.95$, where p_j is the true DLT rate of dose level j. When

the lowest dose is eliminated, stop the dose escalation for safety. The trial design is illustrated in Table 8.c. Dose escalation will continue until the maximum sample size is reached or the number of patients treated at the current dose level reaches 9, and the recommendation is to retain at the current dose level. Isotonic regression method will be used on the cumulative DLT rate for each dose level to determine the MTD, defined as the highest TAK-676 dose in combination with radiation therapy and pembrolizumab that does not result in unacceptable toxicities.

Table 8.c Dose Escalation/De-escalation Rule for the BOIN Design

Number of Patients Treated at the Current Dose	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Escalate if number of DLT ≤	0	0	0	0	1	1	1	1	2
De-escalate if number of DLT ≥	1	1	2	2	2	3	3	3	4
Eliminate if number of DLT ≥	NA	NA	3	3	4	4	5	5	5

BOIN: Bayesian Optimal Interval; DLT: dose-limiting toxicity; NA: not applicable.
Number of DLTs is the number of patients with at least 1 DLT.

Table 8.d Planned Dose Levels of TAK-676

Dose Level	**Dose (unit)**
-2	0.05 mg
-1	0.1 mg
1	0.2 mg
2	0.4 mg
3	0.8 mg
4	1.2 mg
5	1.6 mg
6	2.0 mg
7	2.5 mg
8	3.5 mg
9	5.0 mg
10	7.0 mg
11	9.0 mg

Enrollment into dose levels of TAK-676 in this study may not surpass the highest dose determined to be safe in combination with pembrolizumab in Study TAK-676-1002, the FIH dose escalation study of TAK-676 with or without pembrolizumab in patients with advanced solid tumors.

Additional cohorts at 3.5 mg (~1.4-fold increase from previous dose level), 5.0 mg (~1.4-fold increase), 7.0 mg (~1.4-fold increase), and 9.0 mg (~1.3-fold increase) are proposed. The dose escalation increments (~1.4-fold) were based on the preliminary PK results from the TAK-676-1002 FIH study, which showed modest intersubject variability in clearance and overlapping TAK-676 exposures as measured by area under the concentration-time curve from

time 0 to infinity across cohorts from 0.4 to 1.2 mg on C1D1 or C1D8. Because enrollment in this study may not surpass the highest TAK-676 dose level determined to be safe in combination with pembrolizumab in the FIH study, a more aggressive dose escalation (not to exceed 1 dose level below the highest dose level of TAK-676 that has been determined to be safe in combination with pembrolizumab from the TAK-676-1002 FIH study) is permissible following discussions between the sponsor and the investigators, if supported by evolving safety, tolerability, PK, and pharmacodynamic data of TAK-676 (eg, if this study determines that radiation + pembrolizumab + 0.8 mg TAK-676 is safe and the TAK-676-1002 FIH study has determined that pembrolizumab + 2.5 mg TAK-676 is safe, escalation from 0.8 mg TAK-676 to 2.0 mg TAK-676 will be permissible in this study). Other dose escalation decisions, evaluation of intermediate doses, expansion of an existing dose level, and stopping the dose escalation early are all permissible following discussions between the sponsor and the investigators, if such measures are needed for patient safety, or for a better understanding of the dose-related toxicity, exposure, and/or pharmacodynamics. Other non-DLT safety and available clinical, PK, or biomarker data will also be considered to inform subsequent dose recommendations, dose escalation decisions, and potential MTD/RP2D estimation.

8.4 Dose Modification Guidelines

Decisions regarding which study drug requires dose modification will be dependent upon the toxicity, its onset, and its time course. The causal relationship of any reported events (AEs or SAEs) should be assessed by the investigator in relation to radiation, TAK-676, and pembrolizumab. After discussion between the investigator and the sponsor, alternative dose modifications may be recommended to maximize exposure of study treatment while protecting patient safety. Discussions and agreements will be documented.

General Principles

Treatment with TAK-676 in combination with pembrolizumab following radiation therapy will occur in 21-day cycles. Toxicities are to be assessed according to the NCI CTCAE, Version 5.0. All toxicities that occur during the study will be actively managed following the SOC unless otherwise specified in the protocol. The causal relationship of any reported events (AEs or SAEs) should be assessed by the investigator in relation to radiation therapy, pembrolizumab, and/or TAK-676. Patients experiencing AEs attributed to TAK-676 in combination with pembrolizumab may continue study treatment and maintain the same dose, have doses held, have doses of TAK-676 reduced, or permanently discontinue from the study. Patients who have the TAK-676 dose held due to a treatment-related or possibly related AE may resume study drug after resolution of the AE, and may either maintain the same dose level or have doses of TAK-676 reduced after consultation with the sponsor. When a dose reduction occurs, the TAK-676 dose will be reduced to the next lower dose that has been established as a safe dose during dose escalation. If initial dose adjustment does not provide sufficient relief, the dose of TAK-676 can be further reduced if the treating physician considers that the patient is benefiting from study treatment. If TAK-676 dosing is delayed for more than 21 days for TAK-676–related or possibly related toxicities, despite supportive treatment per standard clinical practice or more than 2 dose reductions are required in a patient, this patient will have study treatment discontinued.

For AEs that occur during the study but are not related to TAK-676 or pembrolizumab, the dose modification of TAK-676 and/or pembrolizumab, in principle, is not required. However, these situations should be discussed with the medical monitor. Depending on medical conditions and the possibility of potential worsening of toxicities by the continued administration of TAK-676 and/or pembrolizumab, investigators can decide to have the TAK-676 and/or pembrolizumab dose held or reduced (in the case of TAK-676) until the resolution of the AE in consultation with the sponsor, as needed.

Dose modification guidelines for toxicities are described in Table 8.f and Table 8.g for TAK-676 based on the nature and severity of AEs and causality determination by investigators. Further clarification can be obtained in consultation with the sponsor. The pembrolizumab dose cannot be reduced; it may only be skipped (ie, patient is not dosed), interrupted (ie, dosing is paused during infusion), or discontinued.

8.4.1 Criteria for Administering a Subsequent Dose/Starting a New Cycle

Treatment with TAK-676 in combination with pembrolizumab will use a cycle length of 21 days. For a subsequent dose of TAK-676 to be administered or a new cycle of treatment to begin, the patient must meet the following criteria:

- ANC ≥1000/µL, platelet count ≥75,000/µL, and hemoglobin ≥8.0 g/dL.
- Total bilirubin ≤1.5 times the institutional ULN. For patients with Gilbert's disease, ≤3 mg/dL.
- Serum ALT and AST ≤3.0 times the ULN (or ≤5.0 times the ULN with presence of liver metastases).
- Albumin ≥3.0 g/dL.
- Estimated creatinine clearance using the Cockcroft-Gault formula ≥30 mL/min.
- Oxygen saturation of ≥92% on room air.

Before administering a subsequent dose or starting a new treatment cycle, TAK-676–related and/or pembrolizumab-related AEs or clinically significant laboratory abnormalities must have returned to Grade ≤1 or baseline, except for alopecia, Grade 2 peripheral neuropathy, and/or autoimmune endocrinopathies with stable endocrine replacement therapy. For clinically significant lab values that occur during the study but are not related to TAK-676 or pembrolizumab, the dose modification of TAK-676 and/or pembrolizumab, in principle, is not required. However, these situations should be discussed and documented with the medical monitor before administering a subsequent dose.

If the patient fails to meet the above-cited criteria for retreatment, treatment should be held (in the case of a subsequent dose) or delayed (in the case of starting a new cycle) by 1 week, at the end of which the patient will be re-evaluated to determine whether the criteria for retreatment have been met. If criteria are subsequently met, the previous dose will be considered "missed" within that cycle. If a dose reduction is considered, TAK-676 will be reduced by 1 dose level, see Section 8.4.

8.4.2 Criteria for Dose Modification of Radiation

If radiation-related AEs are observed that require reduction of the planned radiation schedule, the dose may be reduced in subsequent cohorts following discussion between the investigator and sponsor.

Table 8.e Dose Modification of Radiation

Dose Reduction Levels	Dose Fractionation Scheme	Biologically Effective Dose (BED3) [a]	Biologically Equivalent Dose in 2 Gy Fractions [a]
Planned dose	8 Gy × 3 fractions	88.0 Gy_3	52.8 Gy
(-) 1 dose level	8 Gy × 1 fraction	29.33 Gy_3	17.6 Gy

Source: (Brenner 2008).

Gy: gray.

[a] Biologically equivalent dose of each radiation fractionation scheme based on linear-quadratic model and assuming and α/β of 3. This was selected for normal tissue toxicity concerns given this is primarily a safety study.

8.4.3 Criteria for Dose Modification of Pembrolizumab

Dosing of pembrolizumab cannot be reduced; it can only be skipped, interrupted, or discontinued. Refer to the pembrolizumab prescribing label for recommended dose modification and/or discontinuation guidelines.

8.4.4 Criteria for Dose Modification of TAK-676

Dosing of TAK-676 should be reduced according to the dose modification recommendations listed in Table 8.f for nonhematologic toxicity and Table 8.g for hematologic toxicities. If indicated, TAK-676 dose should be reduced by at least 1 dose level (or by 50% if the patient is receiving the first dose level). For Grade 1 and Grade 2 toxicities, the investigator may consider making a dose adjustment to TAK-676 or pembrolizumab or both agents as clinically indicated by the nature of toxicity. For Grade ≥3 AEs, dose adjustments to both TAK-676 and pembrolizumab must be made as indicated in the following tables and/or prescribing information for pembrolizumab. During this study, further adjustment to dose modification guidelines may be made following discussion with the sponsor and study investigators.

If the initial dose adjustment does not provide sufficient relief, the dose of TAK-676 can be further reduced by another dose level with agreement by the sponsor if the treating physician believes that the patient is receiving benefit. Once a dose is reduced, it will not be re-escalated. However, if further evaluation reveals that the AE that led to the dose reduction was not TAK-676 related, or there were other circumstances contributing to the AE that are unlikely to recur, the dose may be re-escalated to the original dose level after discussion with the sponsor and the treating physician. A patient can have up to 2 dose level reductions of TAK-676 as a single agent or in combination due to AEs, but further reductions are not permitted (the patient should discontinue study drug in this case).

Table 8.f Guidelines for TAK-676 Dose Modification and/or Discontinuation for Nonhematologic Toxicity

Refer to the pembrolizumab prescribing information for recommended dose modification and/or discontinuation guidelines.

NCI CTCAE Grade	TAK-676 Dose Modification
CRS	
Grade 1: Fever with or without constitutional symptoms	• Continue TAK-676 at the same dose level.
Grade 2: Hypotension responding to fluids; hypoxia responding to <40% O_2	• Interrupt TAK-676 until recovers to Grade ≤1. • Once recovered, restart TAK-676 with a dose reduction of 1 dose level. If a second event occurs, permanently discontinue TAK-676. • A maximum of 2 consecutive TAK-676 doses can be skipped; otherwise TAK-676 must be permanently discontinued.
Grade 3: Hypotension managed with 1 pressor; hypoxia requiring ≥40% O_2	• Permanently discontinue TAK-676.
Grade 4: Life-threatening consequences; urgent intervention indicated	• Permanently discontinue TAK-676.
Infusion-Related Reactions	
Grade 2	• Hold TAK-676 infusion until symptoms resolve to Grade ≤1. • Resume at a slower rate. • Consider premedication before the subsequent dose. • Permanently discontinue if recurrent on rechallenging despite premedication use.
Grade 3 or 4	• Permanently discontinue TAK-676.
Colitis	
Grade 2 or 3	• Hold TAK-676 until improvement to a Grade ≤1 or baseline. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.
Grade 4	• Permanently discontinue TAK-676.

Table 8.f Guidelines for TAK-676 Dose Modification and/or Discontinuation for Nonhematologic Toxicity

Refer to the pembrolizumab prescribing information for recommended dose modification and/or discontinuation guidelines.

NCI CTCAE Grade	TAK-676 Dose Modification
Hepatotoxicity	
AST/ALT ≤3 × ULN, or ≤5 × ULN in the case of known liver metastases	• Continue TAK-676 at the same dose level.
AST/ALT >3-5 times the ULN	• Hold TAK-676 until resolution to AST/ALT ≤ 3 × ULN, or ≤5 × ULN in the case of known liver metastases. • Resume at same dose level. • Permanently discontinue if recurrent on rechallenging or concurrent TBILI > 2 × ULN (per Hy's law). • Monitoring of hepatic laboratory tests, including ALT, AST, ALP, TBILI, DBILI, PT-INR is recommended until improved AST/ALT ≤3 × ULN, or ≤5 × ULN in the case of known liver metastases.
AST/ALT >5-10 times the ULN	• Hold TAK-676 until improvement to AST/ALT ≤3 × ULN or ≤5.0 × ULN in the case of known liver metastases. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on re-challenging, or concurrent TBILI > 2 × ULN (per Hy's law). • Monitoring of hepatic laboratory tests, including ALT, AST, ALP, TBILI, DBILI, and PT-INR is recommended until improved AST/ALT ≤3 × ULN or ≤5.0 × ULN in the case of known liver metastases.
AST/ALT >10 times the ULN or Child-Pugh score ≥9	• Permanently discontinue TAK-676.
Elevated bilirubin (Grade 2)	• Hold TAK-676 until resolution to T-Bili ≤1.5 times the ULN or ≤3 mg/dL for patients with Gilbert's disease. • Resume at same dose level. • Reduce by 1 lower dose level if recurrent.
Elevated bilirubin (Grade 3 or 4)	• Permanently discontinue TAK-676.
Endocrinopathies	
Grade 2	• Continue TAK-676 at the same dose level. • Replacement therapy as clinically indicated.
Grade 3 or 4	• Hold TAK-676 until clinically stable. • Resume at 1 lower dose level. • Replacement therapy as clinically indicated.

Table 8.f Guidelines for TAK-676 Dose Modification and/or Discontinuation for Nonhematologic Toxicity

Refer to the pembrolizumab prescribing information for recommended dose modification and/or discontinuation guidelines.	
NCI CTCAE Grade	**TAK-676 Dose Modification**
Increased Creatinine	
Grade 1	• Continue TAK-676 at the same dose and monitor creatinine weekly.
Grade 2	• Hold TAK-676 until resolution to Grade ≤1 or baseline. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.
Grade 3 and 4	• Permanently discontinue TAK-676.
Pneumonitis	
Grade 2	• Hold TAK-676 until resolution to Grade ≤1 or baseline. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.
Grade 3 or 4	• Permanently discontinue TAK-676.
Rash (Skin and Subcutaneous Tissue Disorders)	
Grade 2	• Hold TAK-676 until resolution to Grade ≤1 or baseline. • Resume TAK-676 at same dose level. • Reduce TAK-676 by 1 dose level if recurrent.
Grade 3	• Hold TAK-676 until resolution to Grade ≤1 or baseline. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.
Grade 4	• Permanently discontinue TAK-676.
Other Immune Mediated Adverse Events	
Grade 2	• Hold TAK-676 until resolution to Grade <1. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.
Grade 3 or 4	• Permanently discontinue TAK-676.
Other Nonhematologic Toxicities	
All other Grade 3 nonhematologic toxicities (except for Grade ≥3 nonhematological exceptions outlined in Section 8.2)	• Hold TAK-676 until resolution to Grade ≤1 or baseline. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.

Table 8.f Guidelines for TAK-676 Dose Modification and/or Discontinuation for Nonhematologic Toxicity

Refer to the pembrolizumab prescribing information for recommended dose modification and/or discontinuation guidelines.

NCI CTCAE Grade	TAK-676 Dose Modification
All other Grade 4 nonhematologic toxicities (except for Grade ≥4 nonhematological exceptions outlined in Section 8.2)	• Permanently discontinue TAK-676.

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CRS: cytokine release syndrome; CTCAE: Common Terminology Criteria for Adverse Events; DBILI: direct bilirubin; NCI: National Cancer Institute; PT-INR: prothrombin international normalized ratio; TBILI: total bilirubin; ULN: upper limit of normal.

Table 8.g Guidelines for TAK-676 Dose Modification and/or Discontinuation for Hematologic Toxicity

Refer to the pembrolizumab Prescribing Information for recommended dose modification and/or discontinuation guidelines.

NCI CTCAE Grade	TAK-676 Dose Modification
Neutrophil Count (ANC) Decreased	
Grade 4 lasting >5 days	• Hold TAK-676 until resolved to ≥1000/mm^3 or baseline, in absence of fever/evidence of infection. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.
Febrile neutropenia	• Hold TAK-676 until resolved to ≥1000/mm^3 or baseline, and fever/infection have resolved. • Resume at 1 lower dose level. • Permanently discontinue if recurrent on rechallenging.
Platelet Count Decreased	
Grade 3 without bleeding	• Hold TAK-676 until resolved to ≥75000/mm^3, then: • If spontaneously resolved in ≤7 days, resume at same dose level. • If resolved in >7 days, reduce by 1 dose level.
Grade 4	• Hold TAK-676 until resolved to ≥75000/mm^3, then reduce by 1 dose level. • Permanently discontinue if recurrent on rechallenging.
Platelets <10,000 cells/µL, or thrombocytopenia Grade ≥3 associated clinically significant bleeding	• Permanently discontinue TAK-676.
Anemia	
Grade 3	• Transfuse PRBCs as clinically indicated. • Resume at 1 lower dose level lower.
Grade 4	• Permanently discontinue TAK-676.

Table 8.g Guidelines for TAK-676 Dose Modification and/or Discontinuation for Hematologic Toxicity

Refer to the pembrolizumab Prescribing Information for recommended dose modification and/or discontinuation guidelines.	
NCI CTCAE Grade	**TAK-676 Dose Modification**

ANC: absolute neutrophil count; CTCAE: Common Terminology Criteria for Adverse Events; NCI: National Cancer Institute; PRBCs: packed red blood cells.

8.4.5 Criteria for Discontinuation of TAK-676

Patients who meet criteria for DLT during Cycle 1 will be discontinued from therapy unless the principal investigator believes the potential risks and benefits of a lower TAK-676 dose justifies a rechallenge at a lower dose.

The following events should lead to discontinuation of TAK-676:

1. Nonhematological toxicity:
   a) Grade >3 nonhematological toxicities that do not meet the DLT exception criteria.
   b) Grade ≥3 CRS.
   c) Grade ≥3 infusion-related reactions or recurrent Grade 2 infusion-related reactions.
   d) Grade ≥3 pneumonitis or recurrent Grade 2 pneumonitis despite 1 TAK-676 dose reduction.
   e) Grade ≥3 creatinine increase.
   f) Grade ≥3 immune-mediated AEs.
   g) Grade ≥ 3 bilirubin increase.
   h) ALT and/or AST >10 times the ULN or Child-Pugh score ≥9.
   i) Grade ≥2 drug-related uveitis.
2. Hematological toxicity:
   a) Grade 4 anemia.
   b) Recurrent Grade 4 neutropenia lasting >7 days or febrile neutropenia despite 1 TAK-676 dose reduction.
   c) Recurrent Grade 4 thrombocytopenia despite 1 TAK-676 dose reduction.
   d) Platelets <10,000 cells/μL or thrombocytopenia Grade ≥3 associated clinically significant bleeding.

If more than 2 dose reductions for other AEs are required, or if the subsequent dose or cycle of TAK-676 is delayed for >21 days because of TAK-676–related toxicities, then the patient should have study treatment discontinued unless the investigator considers that the patient will receive

benefit by continuing in the study. If treatment discontinuation is determined, the EOT visit should be completed within 30 (+10) days of the last administration of TAK-676 or before the start of subsequent anticancer therapy, whichever occurs first.

Additionally, the study will not begin until safety and tolerability data is available from the first two dose levels in a parallel ongoing dose escalation study (TAK-676-1002) of pembrolizumab with escalating doses of TAK-676 (0.2 and 0.4 mg IV).

8.4.6 Criteria for Discontinuation of Pembrolizumab

Pembrolizumab will be held or discontinued if any of the criteria described in the package insert have been met.

8.4.7 Stopping Rules

The study will be stopped if 2 fatal AEs related to TAK-676 occur at the same dose levels in Cycles 1 and 2.

The stop will result in an immediate halt in enrollment and may also necessitate the halting of treatment of ongoing patients, depending on the nature and severity of the safety risk. A final decision to terminate the study or a protocol amendment will be made only after a full review of the safety data by the sponsor's Safety Management Team and the investigators.

Based on the emerging safety profile, alternative rules may also be considered following discussions between the sponsor and the investigators.

8.5 Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study:

- Prophylactic use of myeloid growth factors (eg, G-CSF) is not allowed in Cycle 1 during dose escalation. Patients who experience severe (ie, Grade 4) neutropenia or febrile neutropenia in Cycle 1 of dose escalation can be managed with growth factor support, if needed, in accordance with ASCO guidelines and/or institutional practices. G-CSF should not be used in this study in a manner that would either help establish eligibility for the study or support escalation of study drug dose during dose escalation.
- Any investigational agent other than pembrolizumab or TAK-676.
- Any concurrent anticancer therapy, including but not limited to chemotherapy, immunotherapy (except for pembrolizumab), biologic or hormonal therapy (except for endocrine therapy for the treatment of breast cancer).
- Concomitant systemic use of corticosteroids or other immunosuppressive medication, current or within 7 days of administration of investigational drug with the following exceptions:
  - Topical, intranasal, inhaled, ocular, intraarticular corticosteroids.
  - Physiological doses of replacement steroid (eg, for adrenal insufficiency).

    eg,

  - Hydrocortisone: Up to 20 mg daily.
  - Prednisone: Up to 15 mg daily.
  - Dexamethasone: Up to 0.75 mg daily.
  - Fludrocortisone: No limitation on use.
- Any live vaccines while on study.
- COVID-19 vaccinations (including boosters and/or additional doses) given within ±3 days of systemic study treatments. To the extent possible, administration of COVID-19 vaccinations should be avoided during the Cycle 1 DLT window; however, vaccination timing remains at the discretion of the investigator.
- Systemic treatment with any known clinical OATP1B1 and/or OATP1B3 inhibitors including:
  – Atazanavir and ritonavir.
  – Clarithromycin.
  – Cyclosporine.
  – Erythromycin.
  – Gemfibrozil.
  – Lopinavir and ritonavir.
  – Rifampin (single dose).
  – Simeprevir.
  – Remdesivir.

  As the above list is not exhaustive, the investigator should consult the prescribed information for any medication under consideration for use to assess if it is a potent OATP1B1 and/or OATP1B3 inhibitor.
- Smoking.
- Vaping.

If a patient experiences an AE on study and TAK-676 dosing is temporarily interrupted because of that AE or there has been an appropriate washout of TAK-676 (ie, 5 times the projected half-life), the medications listed above and in Appendix I may be used for AE management if there is no appropriate alternative treatment available per the investigator's judgment. This situation will be handled on a case by case basis and requires discussion between the investigator and the medical monitor to assess the relative benefit and risk for a given patient. The discussion will be documented in the study file. Patients should be closely monitored for potential toxicities.

8.6 Permitted Concomitant Medications and Procedures

All concomitant medications (defined as any medication given during the study) including prescription and over-the-counter medications, influenza vaccines, and significant nondrug therapies, such as physical therapy, blood transfusions, oxygen supplementation, etc, should be recorded in the designated electronic case report form (eCRF) from signing of the informed consent form (ICF) through 30 days after the last dose of study drug or the start of subsequent antineoplastic therapy, whichever occurs first. This also includes recording of multivitamins or any folate/folic acid supplementation. Patients must be instructed not to take any medications, including over-the-counter medications and herbal supplements, without first consulting with the investigator.

The following medications and procedures are permitted while the patient is receiving the study drug:

- Topical, intranasal, inhaled steroids (eg, for the treatment of asthma) are permitted in addition to physiological doses of replacement steroid in Section 8.5.
- Patients should be transfused with RBCs and platelets as clinically indicated.
- Patients currently on chronic erythropoietin support for anemia may continue to receive erythropoietin.
- Concomitant treatment with bisphosphonates or denosumab will be allowed for patients with evidence of lytic destruction of bone or with osteopenia, according to the ASCO Clinical Practice guidelines or institutional practice in accordance with the product label, unless specifically contraindicated.
- Local radiation of isolated lesions for palliative intent (for example, pain control) is acceptable provided that the requirement for radiation does not represent a progression of the disease and that the radiated lesion is not a target or biopsied lesion.
- For antiemetics, see Section 8.8.6.

Supportive measures consistent with optimal patient care may be given throughout the study.

8.7 Precautions and Restrictions

Refer to the pembrolizumab prescribing label for information on precautions and restrictions for pembrolizumab administration.

Precautions and requirements for a safe TAK-676 administration are detailed in Section 8.1.3.

TAK-676 was evaluated in different studies with animals, including rats and monkeys. Animal studies do not always predict what happens in humans. It is not known if side effects and risks observed in animals will occur in patients taking TAK-676, or if the severity of these side effects and risks will be the same, less, or greater from what was observed in animal studies.

It is not known what effects TAK-676 has on human pregnancy or development of the embryo or fetus. Female patients participating in this study should avoid becoming pregnant, breastfeeding a

baby, or donating eggs for 120 days after the last dose of TAK-676. Male patients should avoid impregnating a female partner and donating sperm for 120 days after the last dose of TAK-676. Female patients of childbearing potential and male patients will be informed as to the potential risk of conception while participating in this study and will be advised that they must use effective contraception (ie, results in a low failure rate when used consistently and correctly), as specified below. A pregnancy test will be performed on each premenopausal female patient of childbearing potential at screening, on Day 1 of each cycle, and again at treatment discontinuation during the EOT visit. A negative pregnancy test must be documented before the administration of study drug on C1D1.

Female patients must meet 1 of the following criteria:

- Postmenopausal (natural amenorrhea and not due to other medical reasons) for at least 1 year before the screening visit.
- Surgically sterile.
- If they are of childbearing potential, agreeable to practicing the following 1 highly effective method and 1 additional effective (barrier) method of contraception at the same time, from the time of signing of the ICF through 120 days after the last dose of study drug.

Highly Effective Methods	Other Effective Methods (Barrier Methods)
Intrauterine device Hormonal (birth control pills/oral contraceptives, injectable contraceptives, contraceptive patches, or contraceptive implants)	Male condom Diaphragm with spermicide; cervical cap; sponge

- Agreeable to practicing true abstinence, when this is in line with the preferred and usual lifestyle of the patient. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.)

Male patients, even if surgically sterilized (ie, status postvasectomy), must agree to 1 of the following:

- Agree to practice effective barrier contraception (eg, latex condom with a spermicidal agent) during the entire study treatment period and through 120 days after the last dose of study drug.
- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception.)

Before starting treatment, male patients should be advised to seek counseling on sperm storage, and female patients should be advised to seek counseling on egg storage, if these are desired.

It is not known whether TAK-676 passes into breast milk. Mothers should not breastfeed during the entire study treatment period and for 120 days after the last dose of study drug.

Before enrolling patients, patients and their caregivers will be educated on potential symptoms including but not limited to fever, edema, lightheadedness, dizziness, tachypnea, dyspnea, confusion, aphasia, dysphasia, ataxia, or tremor. If patients develop any of these symptoms, they should be instructed to immediately contact the principal investigator who may recommend immediate medical attention. Before study start, principal investigators and patients will agree on a health care facility in the event of an emergent situation.

As an additional safety measure, each patient will be provided a patient emergency card detailing treatment site and investigator contact information, clinical study information. This card is intended to provide relevant contact information should a patient be treated by a nonstudy health care provider or emergency medical services. It will also alert nonstudy health care providers to evaluate patients for the potential risks related to TAK-676 administration and provide them with the contact information of the treating site. This card will also remind patients of concerning signs or symptoms for which they should seek immediate medical attention.

8.8 Management of Clinical Events

Therapies that are required to manage AEs and control cancer symptoms are allowed based on standard clinical practice, unless specifically excluded. Supportive care agents, such as G-CSF, blood products (RBC and platelet transfusions), and pain medications are permitted as needed per ASH/ASCO guidelines or local institutional practice. However, these agents should not be used in this study in a manner that would either help establish eligibility for the study or support escalation of study drug dose during dose escalation. If dose alterations are necessary because of the events detailed below, please refer to Section 8.4.

In the sections below, guidance is provided for the management of some AEs that could be expected based on observations in nonclinical toxicology or because of the mechanism of action of TAK-676, pembrolizumab, or radiation. This guidance is not expected to replace investigator judgment in the management of AEs.

Notably, for any clinically significant Grade ≥3 AEs, referral to an appropriate subspecialist should be considered.

8.8.1 Pulmonary Vasculature Toxicity/Noncardiogenic Pulmonary Edema

TAK-676 administration was associated with pulmonary changes in monkeys (please refer to the IB for details) that could resemble vascular leak or noncardiogenic pulmonary edema in humans.

To monitor for this potential side effect and minimize the clinical risk to patients, the following will be performed for patients enrolled:

- Vital signs will be collected before, during and after administration of TAK-676.
- On TAK-676 dosing days in Cycle 1, an additional postdose physical examination will include an assessment of the respiratory system.
- Oxygenation should be carefully monitored and maintained as clinically indicated. Careful diagnosis and aggressive correction of pulmonary symptoms should occur quickly.

Pulmonary imaging (eg, chest CT) is recommended, and investigators should have a low threshold to scan if the patient experiences any pulmonary or vascular symptoms.

As a general approach to established management of pulmonary toxicity, investigators could refer to "High dose IL-2 (Aldesleukin)-expert consensus on best management practices-2014" with slight modification on oxygen saturation regarding dose hold (see Table 8.h) (Dutcher et al. 2014), ASCO guidelines on management of ICI-related lung toxicity (Brahmer et al. 2018), or local institution guidelines.

Table 8.h Pulmonary Toxicity Clinical Management Recommendations

Issue	Considerations	Management
Pulmonary	Tachypnea/dyspnea	**Typical**
	Diagnose etiology and treat	Oxygen 2-4 L nasal cannula, increasing up to 35% rebreather
	Hypoxic causes: fluid overload, capillary leak, bronchospasm	Reassurance or sedative for anxiety, treat bronchospasm or acidosis if appropriate
	Nonhypoxic causes	Consider holding TAK-676 dose if Grade 2 or higher hypoxia is observed in the setting of pulmonary toxicity
	Anxiety, fever, acidosis	
	Maintain oxygen saturation >92%-95%	**Variation**
		Furosemide
		Bronchodilators
		Monitor bicarbonate

Investigators should differentiate pulmonary vasculature toxicity/noncardiogenic pulmonary edema from other clinical conditions, including capillary leak syndrome. Capillary leak syndrome may be characterized by weight gain, generalized edema, hypotension, and hypoalbuminemia (Dutcher et al. 2014). Dyspnea and hypoxia may also reflect capillary leak and could lead to a requirement for supplemental oxygen to maintain an oxygen saturation of 92% to 95%.

Monitor patients for weight gain, edema, hypotension (including orthostatic changes), and serum albumin levels before TAK-676 treatment and as clinically indicated.

8.8.2 CRS

CRS is a disorder characterized by fever, headache, rash, tachycardia, hypotension, tachypnea, and/or hypoxia caused by the release of cytokines. CRS may be associated with cardiac, hepatic and/or renal dysfunction. Severe CRS complications are life-threatening and frequently require intensive care unit (ICU) care with vasopressor and/or ventilation support.

CRS should be graded following NCI CTCAE, Version 5.0. Treatment guidelines are provided below; management of CRS requires close collaboration with specialists in neurology, pulmonology, cardiology, radiology, and/or critical care medicine, as necessary. Given the potential risk of CRS, the on-site pharmacy must confirm that anti–IL-6 agents, such as tocilizumab, are on site and available should they be needed. In circumstances where tocilizumab is not available, other anti–IL-6 agents (eg, siltuximab) will be considered acceptable treatment for

CRS, under the condition that this is consistent with local institutional guidelines/practice and that the availability of the anti–IL-6 agent(s) is(are) confirmed with the site investigator before subject enrollment.

Investigators should differentiate CRS from other critical clinical conditions, including infusion-related reactions, sepsis, capillary leak syndrome, and hemophagocytic lympho-histiocytosis/macrophage activation syndrome. Fever is an important clinical sign that should raise the suspicion of impending CRS. If fever develops, institutional guidelines for management should be followed and patients should be frequently reassessed for signs of CRS. Strongly consider admission for close observation. For patients experiencing Grade ≥2 CRS, ECG telemetry is recommended. Echocardiogram could be obtained as clinically indicated.

The diagnosis and management of CRS is based on clinical parameters as described in Table 8.i. Ferritin, C-reactive protein, and serum cytokine levels should NOT be used for clinical management decisions.

To further mitigate the risk of CRS during this study, vital signs will be measured frequently during and after infusion of TAK-676. Close monitoring of vital signs and clinical condition will continue if the patient experiences symptoms that could be consistent with CRS. If a patient experiences a temperature ≥100.4°F (38°C) at any time on study, they will be instructed to call the principal investigator immediately.

Should symptoms consistent with CRS develop, see Table 8.f for TAK-676 dose modification. If TAK-676 is held, pembrolizumab will also be held until TAK-676 is resumed, or if TAK-676 is discontinued, pembrolizumab will be held until the CRS event recovers to Grade ≤1.

Should TAK-676 be discontinued/held for an AE, continued administration of pembrolizumab is permitted per investigator's discretion and with sponsor's agreement.

Table 8.i NCCN Guidelines Version 3.2021: Management of CAR T Cell–Related Toxicities

CRS [a, b]

- **Prompt and urgent intervention to prevent progression of CRS is required; however, other causes of systemic inflammatory response should be ruled out, including infection and malignancy progression. Empiric treatment for infection is warranted in the neutropenic patient (NCI CTCAE, Version 5.0).** Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading. [c]
- Fever is defined as temperature >38°C not attributable to any other cause. In patients who have CRS then receive antipyretics or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

CRS Grade	Anti–IL-6 Therapy	Corticosteroids [d, e]	Additional Supportive Care
Grade 1: Fever (≥38°C)	For prolonged CRS (>3 days) [f] in patients with significant symptoms and/or comorbidities, consider 1 dose of tocilizumab 8 mg/kg IV over 1 hour (not to exceed 800 mg). [g]	For idecabtagene and lisocabtagene, consider dexamethasone 10 mg IV every 24 hours for early-onset CRS (<72 hours after infusion).	• Empiric broad-spectrum antibiotics, consider GCSF if neutropenic. [h] • Maintenance IV fluids for hydration. • Symptomatic management of organ toxicities.
Grade 2: Fever with hypotension not requiring vasopressors and/or hypoxia [i] requiring low-flow nasal cannula [j] or blow-by.	Tocilizumab 8 mg/kg IV over 1 hour (not to exceed 800 mg/dose). [d] Repeat in 8 hours if no improvement; no more than 3 doses in 24 hours, with a maximum of 4 doses total.	For persistent refractory hypotension after 1-2 doses of anti–IL-6 therapy: Dexamethasone 10 mg IV every 12-24 hours depending on product [k, l]	• IV fluid bolus as needed. • For persistent refractory hypotension after 2 fluid boluses and anti–IL-6 therapy, start vasopressors, consider transfer to ICU, consider echocardiogram, and initiate other methods of hemodynamic monitoring. • Manage per Grade 3 if no improvement within 24 hours after starting anti–IL-6 therapy. • Symptomatic management of organ toxicities.
Grade 3: Fever with hypotension requiring a vasopressor with or without vasopressin and/or hypoxia requiring high-flow cannula,[f] face	Anti–IL-6 therapy per Grade 2 [d] if maximum dose not reached within 24-hour period.	Dexamethasone 10 mg IV every 6 hours. [k] If refractory, manage as Grade 4.	• Transfer to ICU, obtain echocardiogram, and perform hemodynamic monitoring. • Supplemental oxygen. • IV fluid bolus and vasopressors as needed. • Symptomatic management of organ

Table 8.i NCCN Guidelines Version 3.2021: Management of CAR T Cell–Related Toxicities

CRS [a, b]

- **Prompt and urgent intervention to prevent progression of CRS is required; however, other causes of systemic inflammatory response should be ruled out, including infection and malignancy progression. Empiric treatment for infection is warranted in the neutropenic patient (NCI CTCAE, Version 5.0).** Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading. [c]
- Fever is defined as temperature >38°C not attributable to any other cause. In patients who have CRS then receive antipyretics or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

CRS Grade	Anti–IL-6 Therapy	Corticosteroids [d, e]	Additional Supportive Care
mask, nonrebreather mask, or Venturi mask.			toxicities.
Grade 4: Fever with hypotension requiring multiple vasopressors (excluding vasopressin) and/or hypoxia requiring positive pressure (eg, CPAP, BiPAP, intubation, and mechanical ventilation).	Anti–IL-6 therapy as per Grade 2 [d] if maximum dose not reached within 24-hour period.	Dexamethasone 10 mg IV every 6 hours [m]. If refractory, consider 3 doses of methylprednisolone 1000 mg/day IV; if refractory, consider dosing every 12 hours. [m, n]	• ICU care and hemodynamic monitoring. • Mechanical ventilation as needed. • IV fluid bolus and vasopressors as needed. • Symptomatic management of organ toxicities.

BiPAP: bilevel positive pressure therapy; CAR: chimeric antigen receptor; CPAP: continuous positive pressure therapy; CRS: cytokine release syndrome; CTCAE: Common Terminology Criteria for Adverse Events; GCSF: granulocyte colony-stimulating factor; HLH/MAS: hemophagocytic lympho-histiocytosis/macrophage activation syndrome; ICU: intensive care unit; IV: intravenous; NA: not applicable; NCCN: National Comprehensive Cancer Network.

[a] For HLH/MAS during CRS, treat per CRS with addition of steroids. If symptoms do not improve within 48 hours, consider etoposide and intrathecal cytarabine for neurotoxicity.

[b] With permission from Elsevier (Lee et al. 2019).

[c] Organ toxicities should receive a thorough workup and appropriate management.

[d] After each dose, assess need for subsequent dosing.

[e] Antifungal prophylaxis should be strongly considered in patients receiving steroids for the treatment of CRS and/or neurotoxicity.

[f] For axicabtagene ciloleucel or brexucabtagene autoleucel, can consider tocilizumab if CRS symptoms persist for >24 hours.

[g] For lisocabtagene maraleucel, consider tocilizumab for Grade 1 CRS that develops <72 hours after infusion and consider adding dexamethasone 10 mg × 1. For CRS developing ≥72 hours after infusion, treat symptomatically.

Table 8.i NCCN Guidelines Version 3.2021: Management of CAR T Cell–Related Toxicities

CRS [a, b]

- **Prompt and urgent intervention to prevent progression of CRS is required; however, other causes of systemic inflammatory response should be ruled out, including infection and malignancy progression. Empiric treatment for infection is warranted in the neutropenic patient (NCI CTCAE, Version 5.0).** Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading. [c]
- Fever is defined as temperature >38°C not attributable to any other cause. In patients who have CRS then receive antipyretics or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

CRS Grade	Anti–IL-6 Therapy	Corticosteroids [d, e]	Additional Supportive Care

[h] Granulocyte-macrophage colony-stimulating factor is not recommended in the setting of CAR T-cell therapy. A Food and Drug Administration biosimilar is an appropriate substitute for filgrastim.

[i] CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with a temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as Grade 3 CRS.

[j] Low-flow nasal cannula is defined as oxygen delivered at ≤6 L/min. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at >6 L/min.

[k] Alternative steroids at an equivalent dose may be considered.

[l] For axicabtagene ciloleucel, consider dexamethasone 10 mg IV every 24 hours after initial tocilizumab dosing, regardless of clinical response to tocilizumab. For lisocabtagene maraleucel, consider dexamethasone 10 mg IV every 12-24 hours if early-onset CRS. For idecabtagene vicleucel, consider dexamethasone 10 mg IV every 12-24 hours.

[m] For example, methylprednisolone IV 1000 mg/day for 3 days, followed by rapid taper at 250 mg every 12 hours for 2 days, 125 mg every 12 hours for 2 days, and 60 mg every 12 hours for 2 days.

[n] Other agents such as anakinra, siltuximab, ruxolitinib, or extracorporeal cytokine adsorption with CRRT might be considered. Reported experience with these agents is limited.

8.8.3 Infusion-Related Reactions

An infusion-related reaction is a disorder characterized by an adverse local or general response from exposure to an allergen. An infusion-related reaction typically occurs during the infusion or shortly thereafter.

Although TAK-676 is not a biologic agent, its immune-activating properties may produce AEs in the category of infusion-related reactions. If an infusion-related reaction were to occur, it could present as fever, chills, rigors, headache, rash, flushing, pruritus, arthralgias, hypotension or hypertension, bronchospasm, or other symptoms.

Treatment and monitoring of patients until symptoms resolve should be consistent with institutional standards and guidelines, as appropriate. Infusion-related reactions should be diagnosed and managed following institutional guidelines and graded following NCI CTCAE, Version 5.0. Table 8.f provides indications for dose modifications after an infusion-related reaction event.

The patient should be closely monitored until recovery of symptoms. The patient will be permanently discontinued from the study in case of a Grade 3 or 4 life-threatening reaction. All Grade 3 or 4 infusion-related reactions should be reported within 24 hours to the medical monitor and communicated as an SAE if criteria are met (see Section 10.1.3). Concomitant medications administered for infusion-related reaction treatment should be collected in the eCRF. If a patient develops signs and symptoms compatible with infusion-related reactions, and at investigator discretion, premedication can be instituted for the rest of the treatment.

Should emergency treatment be required in the event of life-threatening hypersensitivity or other acute infusion-related reaction, supportive therapy such as oxygen, bronchodilators, epinephrine, antihistamines, and corticosteroids should be given according to local institutional guidelines.

8.8.4 Infusion Site Care

Lesions at the injection site, which may include inflammation or necrosis, represent a potential risk and were observed as microscopic changes only in a non-GLP experiment in rats. Local institutional guidelines must be applied to stress proper administration and prevention of accidental extravasation of TAK-676. Usage of an IV port or central access is highly recommended. The IV line should be flushed as directed in the Pharmacy Manual at the end of each infusion accordingly to local procedures. Patients should be instructed to report any discomfort, pain, or swelling at the infusion site. Monitoring at the beginning and during the infusion for any discomfort, pain, or swelling must be ensured. If extravasation occurs, the infusion must be discontinued immediately, and supportive measures or institutional guidelines applied. There is no known antidote to TAK-676.

8.8.5 Anemia, Thrombocytopenia, and/or Neutropenia

To monitor for side effects of bone marrow toxicity in patients treated with TAK-676, complete blood counts will be monitored regularly for all patients. If anemia, thrombocytopenia, or

neutropenia occur, TAK-676 dose will be modified according to Table 8.g. Precautionary measures should be taken to prevent bleeding and overwhelming infections. Prophylactic use of myeloid growth factors should be avoided during the first cycle of dose escalation. Transfusion and use of growth factors as necessary to manage anemia, thrombocytopenia, and neutropenia are permitted at the discretion of the investigator, consistent with ASH/ASCO guidelines and institutional recommendation. See Section 8.3 for additional guidance.

8.8.6 Nausea and/or Vomiting

This study will not initially employ prophylactic antiemetics before the first dose of the study drug during dose escalation. However, a patient who develops nausea or vomiting will be actively managed by employing optimal antiemetic treatment based on local standard practice. Additionally, antiemetics may be used prophylactically as clinically indicated following the occurrence of a first event of study drug–related or possibly related nausea and/or vomiting. An optimal antiemetic regimen is defined as one that employs a neuroleptic, a 5-HT_3 serotonin receptor antagonist, or an NK-1 antagonist may be added. Steroid use should be avoided unless it is a clinical necessity. If the nausea/vomiting is thought to be immune mediated, management should follow evolving consensus guidelines for management of immune mediated toxicities (Brahmer et al. 2021; Brahmer et al. 2018).

8.8.7 Diarrhea

This study will not initially employ prophylactic antidiarrheals. However, there is no prohibition against their use in the management of a patient who develops diarrhea. Patients will be instructed to take antidiarrheal medication(s) at the physician's discretion until they are diarrhea-free for at least 12 hours. Fluid intake should be maintained to avoid dehydration. If the diarrhea is thought to be immune mediated or immune-mediated colitis is suspected, management should follow evolving consensus guidelines for management of immune mediated toxicities (Brahmer et al. 2021; Brahmer et al. 2018).

8.8.8 Skin Conditions/Rash

It is recommended that for all severity grades of cutaneous events, the diagnostic work-up should include:

- Evaluation of a pertinent history and physical examination.
- Ruling out any other etiology of the skin problem (including infection, another drug side effect, a skin condition linked to another systemic disease or unrelated primary skin disorder).
- Evaluation of laboratory values, including a blood cell count, liver and kidney tests.
- Directed serologic studies if an autoimmune condition is suspected.
- Skin biopsy, consultation with dermatologist if indicated.
- Review full list of patient medications.

Further management of any noted skin toxicities should follow local management guidelines and evolving consensus guidelines for management of potentially immune-mediated skin toxicities (Brahmer et al. 2018).

8.8.9 Fluid Deficit

Fluid deficit should be corrected before initiation of study drug and during treatment. Patients on study should be advised to maintain adequate oral hydration.

8.8.10 Management of Pembrolizumab Immune-Mediated AEs

Pembrolizumab can cause immune-mediated reactions, including pneumonitis, colitis, hepatitis, endocrinopathies, nephritis, skin adverse reactions, and infusion-related reactions. Monitoring of these AEs is required through 90 days after the last dose of study drug or the start of subsequent anticancer therapy, whichever occurs first. Patients with AEs that are suspected to be related to pembrolizumab should be evaluated by appropriate methodology, including physical examinations, laboratory tests, and imaging. Dosing of pembrolizumab should be interrupted based on the guidance in Section 8.4. Treatment of these AEs should follow evolving consensus guidelines for management of potential immune CPI-related AEs (Brahmer et al. 2018) and will be based on SOC, local institutional guidance, the WARNINGS AND PRECAUTIONS section of the package insert, and the proper use guidance of pembrolizumab.

8.8.11 Management of Radiation-Associated AEs

There is a potential for synergistic toxicity with combination use of radiation and pembrolizumab with or without TAK-676. Increased inflammation or radiation burn in the radiation field will be treated with local measures as per institutional guidelines. Antibiotics (local or systemic) may be used if there is a clinical suspicion of infection.

8.8.12 Management of COVID-19–Positive Patients

A consensus plan of action has been developed for the management of patients within the context of the COVID-19 pandemic. During the screening period, a patient who exhibits symptoms consistent with COVID-19 (eg, fever, shortness of breath, sore throat) will undergo COVID-19 testing as per institutional guidelines. A patient with a negative test result will undergo further diagnostic work-up and management at the discretion of the site investigator. If a patient tests positive for COVID-19 during the screening period, enrollment should be paused during which time the investigator, in consultation with the sponsor, will determine the appropriate course of action for that patient. For patients with symptoms and/or requiring treatment, symptoms should have resolved to baseline and treatment should be completed at least 7 days before enrollment (a negative test for severe acute respiratory syndrome coronavirus-2 [SARS-CoV-2] by polymerase chain reaction (PCR) may also be documented if required by local institutional guidelines). Asymptomatic patients who test positive should wait at least 7 days from the positive test result to ensure they continue to remain asymptomatic before enrolling.

While on study treatment, a patient who exhibits symptoms consistent with COVID-19 (eg. fever, shortness of breath, sore throat) will undergo COVID-19 testing as per institutional guidelines, in addition to work-up for pulmonary toxicity related to the trial intervention. A patient with a negative test result will undergo further diagnostic work-up and management at the discretion of the site investigator. If a patient tests positive for COVID-19 while on study, treatment should be held (up to 21 days), during which time the sponsor and investigator will determine the appropriate course of action for that patient. Suggested guidance is as follows:

- For patients with symptoms and/or requiring treatment, symptoms should have resolved to baseline and treatment should be completed at least 7 days before resuming study drug(s) (a negative test for SARS-CoV-2 by PCR may also be documented if required by local institutional guidelines).
- Asymptomatic patients who test positive should wait at least 7 days from the positive test result to ensure they continue to remain asymptomatic before resuming study drug(s).

In all cases, consultation with the medical monitor should also be considered, as appropriate.

8.9 Blinding and Unblinding

This is an open-label study.

8.10 Description of Investigational Agents

8.10.1 TAK-676

TAK-676 drug substance is a white-to-off white powder and freely soluble in aqueous solution. "TAK-676 Injection 3 mg/3 mL, as free base" is supplied as a single-use, 17 cc stoppered glass vial with an aluminum flip-off seal, providing 3 mL (3 mg) of TAK-676 drug substance solution as free base. The excipients are listed in the table below.

Ingredient	Function
Trisodium citrate dihydrate	Buffer
Citric acid monohydrate	Buffer
Sodium chloride	Tonicity agent

For specific information about the storage and handling of TAK-676 drug product, refer to the study or Pharmacy Manual associated with this study protocol.

8.10.2 Pembrolizumab

"Pembrolizumab Injection 100 mg/4 mL (25 mg/mL) solution in a single-dose vial" will be either labeled as study material and supplied by the sponsor or sourced locally by the clinical site when arrangements have been made and agreed to by sponsor and where regulations allow for clinical site sourcing, appropriate labeling, and compliance with local and regional regulations. For preparation, handling, and administration instructions related to pembrolizumab, please consult

the Pharmacy Manual and/or the product information included with pembrolizumab (eg, package insert).

8.10.3 Radiation Therapy

For details on radiation therapy, refer Appendix J.

8.11 Preparation, Reconstitution, and Dispensation

8.11.1 TAK-676

The drug product will be administered by IV infusion over 60 ±10 minutes. After the end of the infusion, the IV line should be flushed as directed in the Pharmacy Manual. Detailed dosage preparation instructions are provided in the Directions for Use located in the Pharmacy Manual.

In the absence of compatibility studies, this medicinal product must not be mixed with other medicinal products.

TAK-676 is an anticancer drug, and as with other potentially toxic compounds, caution should be exercised when handling TAK-676.

Study drug products should be inspected visually for particulate matter and discoloration before administration, whenever solution and container permit.

8.11.2 Pembrolizumab

Please refer to the Pharmacy Manual and/or the most recent pembrolizumab prescribing information as applicable to the specific country for preparation, reconstitution, and administration instructions.

8.12 Packaging and Labeling

8.12.1 TAK-676

"TAK-676 Injection 3 mg/3 mL, as free base" will be labeled as investigational drug product. All label information will fulfill requirements specified by local governing regulations. Additional details are provided in the Pharmacy Manual.

8.12.2 Pembrolizumab

Pembrolizumab, if provided by sponsor, will be labeled as investigational drug product. All label information will fulfill requirements specified by local governing regulations. Additional details are provided in the Pharmacy Manual.

8.13 Storage, Handling, and Accountability

8.13.1 TAK-676

Complete receipt, inventory, accountability, reconciliation, and destruction records will be maintained by authorized personnel at the study site for all used and unused study drug vials. A drug dispensing log, including records of drug received from the sponsor and drug dispensed to patients will be provided and kept at the study site. For all study sites, the local country sponsor personnel or designee will provide appropriate documentation that must be completed for study drug accountability, return, and destruction. Instructions are provided in the Pharmacy Manual.

TAK-676 must be stored in a secure, limited-access location, according to the conditions specified on the drug label and remain in the original carton until dispensed. The investigator or designee must confirm that appropriate temperature conditions have been maintained and that any temperature excursions are reported and resolved before use. Please refer to the Pharmacy Manual for additional instructions.

8.13.2 Pembrolizumab

Pembrolizumab must be stored in a secure, limited-access location, according to conditions specified on the drug label. The investigator or designee must confirm that appropriate temperature conditions have been maintained and that any excursions are reported and resolved before use. Refer to the Pharmacy Manual and/or the most recent pembrolizumab prescribing information as applicable to the specific country for additional details.

8.14 Other Protocol-Specified Materials

Information on supplies required by the site for drug administration is provided in the Pharmacy Manual. Clinical supplies other than study drug to be provided by the sponsor or designee are specified in the Study Manual.

9.0 STUDY CONDUCT

This trial will be conducted in compliance with the protocol, GCP, applicable regulatory requirements, and ICH guidelines.

9.1 Study Personnel and Organizations

The contact information for the project clinician for this study, the central laboratory and any additional clinical laboratories, the coordinating investigator, and other vendors participating in the study can be found in the Study Manual.

For 24-hour contact information, please refer to the Study Manual or equivalent.

9.2 Arrangements for Recruitment of Patients

Recruitment and enrollment strategies for this study may include recruitment from the investigator's local practice or referrals from other physicians. If advertisements become part of

the recruitment strategy, they will be reviewed by the institutional review board (IRB)/ independent ethics committee (IEC).

9.3 Treatment Group Assignments

This is not a randomized study. Patients will be assigned to a dose cohort based on the dose escalation rules described in Section 8.3. The ongoing TAK-676-1002 FIH study will also provide clinical data on the safety and tolerability of TAK-676 + pembrolizumab. In the combination arm of the FIH study, TAK-676 is administered in a dose escalating fashion (starting at 0.2 mg IV on Days 1, 8, and 15 of a 21-day cycle) along with pembrolizumab at the fixed dose of 200 mg IV every 3 weeks. Safety data was to be obtained from at least 2 dose cohorts of the FIH combination arm before administering TAK-676 with pembrolizumab following radiation therapy in the current study.

Enrollment into dose levels of TAK-676 may not surpass the highest dose determined to be safe in Study TAK-676-1002, the FIH dose escalation study of TAK-676 with or without pembrolizumab in patients with advanced solid tumors.

9.4 Study Procedures

Refer to the SOEs (Appendix A) for timing of assessments. Additional details are provided as necessary in the sections that follow. Evaluations during the screening period are to be conducted within 28 days before the first dose of radiation unless otherwise specified. Unless otherwise noted, evaluations during the treatment period must occur before drug administration on scheduled visits. Tests and procedures should be performed on schedule for all visits. The timing of PK and pharmacodynamic assessments is specified in the SOE (Appendix A). Refer to the SOE (Appendix A) for timing of assessments. Additional details are provided as necessary in the sections that follow.

9.4.1 Informed Consent

Each patient must provide written informed consent before any study-required procedures are conducted, unless those procedures are performed as part of the patient's standard care.

9.4.2 Patient Demographics

The date of birth, race, ethnicity, and sex of the patient are to be recorded during screening.

9.4.3 Medical History

During the screening period, a complete medical history, including smoking/vaping history, will be compiled for each patient. The history will emphasize the background and progress of the patient's malignancy and include a description of prior therapies for it and the best response achieved by each one. In addition, concomitant medications will be recorded as specified in Section 9.4.9.

Available tumor genomic information obtained at the site will be collected.

9.4.4 Physical Examination

Physical examinations will be completed per SOC at the times specified in the SOE (Appendix A). Complete physical examinations will include an assessment of the following: skin, head, eyes, ears, nose, throat, respiratory system, cardiovascular system, GI system, neurological condition, blood and lymphatic systems, and musculoskeletal system.

Symptom-directed physical examination will be conducted on Day 1 of each cycle before administration of the first dose of study treatment and any other time point during the cycle based on clinical need.

On dosing days in Cycle 1, an additional post dose physical examination will include an assessment of the respiratory and neurologic systems.

9.4.5 Patient Height and Weight

Height will be measured only during screening. Body weight will be measured at the times specified in the SOE (Appendix A) and as clinically indicated.

9.4.6 ECOG Performance Status

Performance status is to be assessed using the ECOG scale (see Appendix D for a description of the scale) at the times specified in the SOE (Appendix A).

9.4.7 Vital Signs

Vital sign measurements, including diastolic and systolic blood pressure while sitting (after approximately 5 minutes in this position), heart rate, respiratory rate, oxygen saturation, and temperature, will be assessed as specified in the SOE (Appendix A). Refer to Section 8.1.3 for details on vital sign measurements during and post pembrolizumab and TAK-676 infusions.

Vital signs should be measured and recorded any time the patient develops symptoms associated with potential risks of TAK-676 and/or pembrolizumab.

9.4.7.1 Pulse Oximetry

Pulse oximetry should be obtained before each dose of TAK-676 and at any time a patient has any new or worsening respiratory symptoms. A reading at rest and on exertion if clinically indicated (during waking hours) should be obtained. The extent of the exertion should be based on the judgment of the investigator, but should remain consistent for each individual patient throughout the study.

If the patient's status changes, the investigator can alter the extent of exertion based on their medical judgment. If a patient shows changes in pulse oximetry or other pulmonary-related signs (eg, hypoxia, fever) or symptoms (eg, dyspnea, cough, fever) consistent with possible pulmonary AEs, the patient should be immediately evaluated to rule out pulmonary toxicity.

9.4.8 Pregnancy Test

The pregnancy test must be a beta-human chorionic gonadotropin test, and either urine or serum can be used. Women who are not of childbearing potential (status posthysterectomy, status post-bilateral oophorectomy, or postmenopausal [defined as amenorrhea for at least 12 months]) and men do not need to have the test performed.

Women of childbearing potential must have a confirmed negative pregnancy test at screening (serum or urine test) and with negative results available before the first dose may be administered. A serum or urine pregnancy test must also be completed before the first dose of radiation, on Day 1 of each cycle and at the EOT assessment. If a serum pregnancy test is performed within 3 days from the first dose of radiation and the result is negative, the pregnancy test on the first day of radiation may be waived.

9.4.9 Concomitant Medications and Procedures

Medications used by the patient and therapeutic procedures completed by the patient will be recorded in the eCRF from the time of informed consent signature through 30 days after the last dose of study drug or the start of subsequent systemic antineoplastic therapy, whichever occurs first. See Section 8.5 and Section 8.6 for a list of medications and therapies that are prohibited or allowed during the study.

9.4.10 AEs

Monitoring of all AEs, including SAEs, will be collected throughout the study as specified in the SOE (Appendix A). Refer to Section 10.0 for details regarding definitions, documentation, and reporting of AEs and SAEs.

9.4.11 Enrollment

Enrollment is defined as the time of initiation of the first dose of radiation. Procedures for completing enrollment information are described in the Study Manual.

9.4.12 Cardiac Monitoring

9.4.12.1 12-Lead ECGs and LVEF

The 12-lead standard safety ECGs will be performed to assess eligibility and throughout the study as specified in the SOE (Appendix A). Safety ECGs will be compared with baseline screening ECGs. ECG assessments are to be performed with the patient supine and rested for 5 minutes. A qualified person will interpret the ECGs locally. Additional ECGs may be obtained as clinically indicated at any time during the study at the discretion of the investigator. When the timing of ECG or vital signs measurements coincides with the timing of a blood draw (eg, PK sample), the ECG measurements and vital signs measurements should be completed first, followed by blood sampling. The frequency and timing of ECG collection for safety may be modified based on emerging data and agreement between sponsor and investigators.

The assessment of LVEF measured by echocardiography or MUGA will be performed at screening and as clinically indicated. Any findings from LVEF determinations will be captured as AEs if, in the opinion of the investigator, there has been a clinically significant change from baseline.

9.4.13 Clinical Laboratory Evaluations

Clinical laboratory evaluations will be performed locally. Handling and shipment of clinical laboratory samples will be outlined in the Study Manual.

During the COVID-19 public health emergency, if a trial participant cannot access a clinical trial site for unscheduled bloodwork collection as requested by the investigator, alternative Clinical Laboratory Improvement Amendments-certified sites may be used for laboratory tests that focus on the safety of trial participants. Every effort should be made by the treating site to ensure laboratory normal ranges are collected from any such alternative locations.

9.4.13.1 Clinical Chemistry, Hematology, Coagulation, Thyroid Function, and Urinalysis

Blood samples for analysis of the clinical chemistry, hematology, coagulation, and thyroid function shown in Table 9.a and urine samples for analysis of the parameters shown in Table 9.b will be obtained as specified in the SOE (Appendix A).

Table 9.a Clinical Chemistry, Hematology, Coagulation, and Thyroid Function Tests

Hematology	**Serum Chemistry**	
Hematocrit	Protein (total)	Magnesium
Hemoglobin	Albumin	Phosphate
Leukocytes with differential	Alkaline phosphatase	Potassium
Neutrophils (ANC)	Alanine aminotransferase	Sodium
Platelets (count)	Aspartate aminotransferase	Chloride
RBC	Bilirubin (total)	Urate
MCH	Bilirubin (direct)	
MCV	Gamma glutamyl transferase	
MCHC	Glucose (random blood glucose)	
	Lactate dehydrogenase	
	Amylase	
	Lipase	
	Calcium	
	Bicarbonate (if available as a part of blood chemistry panel of local laboratory)	
	C-reactive protein	
	Blood urea nitrogen	
	Creatinine	
Thyroid Function	**Coagulation**	
Thyrotropin	Activated partial thromboplastin time	
Thyroxine, free (T4)	Prothrombin time (or prothrombin international normalized ratio)	
Total or free triiodothyronine (total or free T3)		
Other		
Ferritin		
Creatine phosphokinase		

ANC: absolute neutrophil count; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; RBC: red blood cell.

Table 9.b Clinical Urinalysis Tests

Urinalysis	
Bilirubin	pH
Glucose	Protein
Ketones	Specific gravity
Leukocyte esterase	Turbidity and color
Nitrite	Urobilinogen
Occult blood	

If creatinine clearance is to be estimated, the Cockcroft-Gault formula (see Appendix F) will be employed as follows (Cockcroft and Gault 1976):

$$Estimated\ creatinine\ clearance = [(140 - age) * weight\ (kg)\ ] / [72 * serum\ creatinine(mg/dL)]$$

For female patients, the result of the formula above should be multiplied by 0.85.

9.4.14 Disease Assessment

For all patients, radiographic assessments of the disease will be performed at screening/baseline (within 28 days before the first study radiation treatment), once at the end of Cycle 2 (Day 15 to 22 [predose]), then at the end of every 3 cycles (Day 15 to 22 [predose]) for the first year, and then at the end of every 6 cycles (+7 days) thereafter until PD or the start of alternative therapies.

Tumor assessments for all patients will continue as scheduled per protocol even if dosing is interrupted. If the patient has had an appropriate CT or MRI scan performed within 28 days of the first dose of radiation the results of that scan may be used for tumor lesion measurements at screening. Patients will undergo CT with IV contrast or MRI, as appropriate to monitor and assess response status, using RECIST (v.1.1) criteria and modified itRECIST criteria (where intratumoral therapy references are largely replaced with radiation therapy).

For this study, CT and/or MRI scans should be acquired with IV contrast unless contraindicated. CT scans of the neck (required for patients with head and neck squamous cell carcinoma and if clinically indicated for patients with TNBC and NSCLC), chest, abdomen, and pelvis will be obtained at screening. Before radiation therapy, the radiology oncologist and medical oncologist should agree on the target lesions identified for irradiation using the baseline imaging assessments. The imaging modalities used and anatomy scanned at baseline should remain consistent throughout the study. If contrast enhanced CT scans are contraindicated for a particular patient, a noncontrast CT of the chest, in addition to contrast enhanced abdomen and pelvis by MRI should be acquired, if possible. Anatomical measurements (summed across target lesions) will be collected at baseline and each subsequent evaluation. In addition, nonmeasurable disease and new lesions will be documented and their statuses evaluated. X-rays and bone scans should be collected as clinically indicated. Radiographic images will be maintained at the site and can be requested by

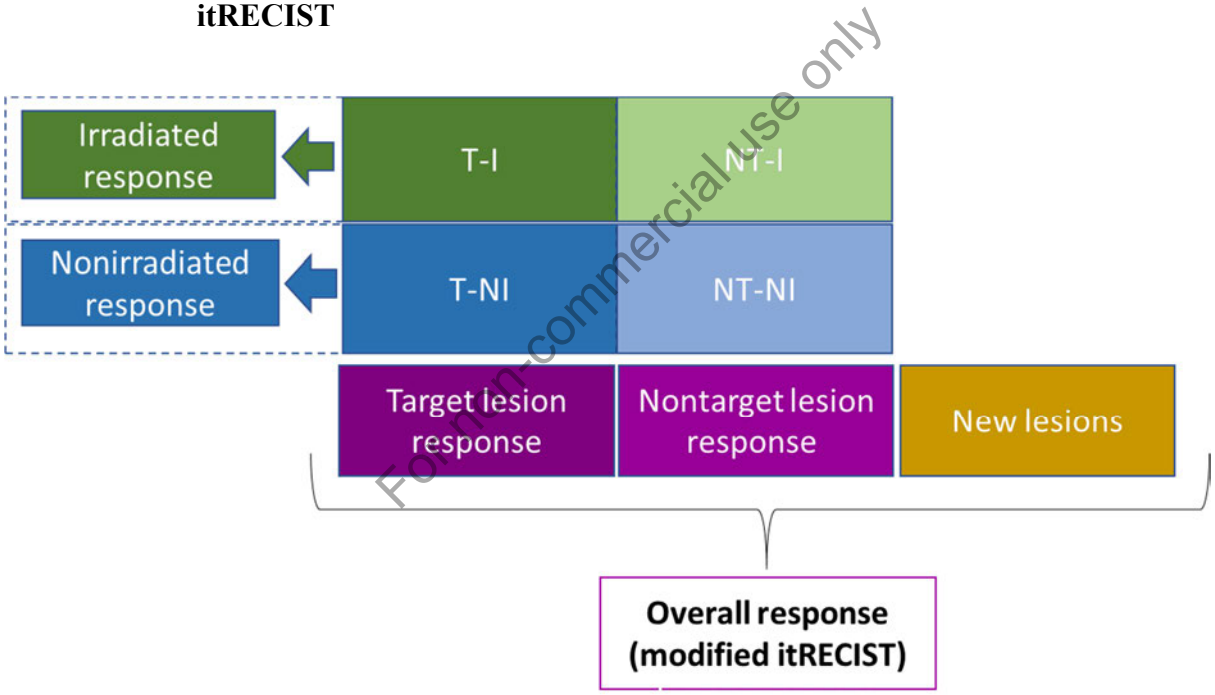
the sponsor for centralized review of the images. Determination of disease status will be based on local investigator assessment.

9.4.14.1 Special Allowances to RECIST v.1.1 and Treatment Beyond Progression

9.4.14.1.1 Irradiated and Nonirradiated Response

In order to meet the secondary objective of this study, investigators (or designee) are asked to not only assess overall response at each timepoint using RECIST v.1.1, but also to assess both the irradiated and nonirradiated response at each timepoint as depicted in Figure 9.a aligning with principles akin to those of itRECIST (where intratumoral therapy references are largely replaced with radiation therapy) (Goldmacher et al. 2020).

Figure 9.a Illustration of Irradiated, Nonirradiated, and Overall Responses per Modified itRECIST



Source: (Goldmacher et al. 2020).
TI: target irradiated; T-NI: target nonirradiated; NT-I: nontarget irradiated; NT-NI: nontarget nonirradiated

According to the inclusion criteria, patients must have at least 2 measurable lesions (ie, >10 mm longest diameter for extranodal lesions, >15 mm short axis for lymph nodes), with at least one inside and at least one other outside of the radiation field. As stated in Section 8.1.1, 1 lesion and up to 3 lesions may be radiated. For the purposes of response assessment, investigators are asked to evaluate response using both RECIST v.1.1 and modified itRECIST.

The principles of RECIST v.1.1 still apply whereby selection of target lesions in a previously irradiated area or in an area subjected to other loco-regional therapy, unless there has been demonstrated progression in the lesion(s), is discouraged; therefore, irradiated lesions in this study will not be considered part of the RECIST v.1.1 assessment. No more than 5 target lesions in total and no more than 2 per organ may be selected in accordance with RECIST v.1.1. The same lesions identified as target and nontarget at screening, before radiation administration, will be followed at each subsequent response assessment.

For response assessment using modified itRECIST, lesion measurements should be performed per RECIST v.1.1 per guidelines on size and reproducibility, except that it is acceptable to select the irradiated lesions as target lesions. Baseline lesions are categorized as target irradiated (T-I), target, nonirradiated (T-NI), nontarget irradiated (NT-I), and nontarget nonirradiated (NT-NI) according to an algorithm (Figure 9.a). The same target and nontarget lesions selected as part of the RECIST v.1.1 assessment at screening may be used for the nonirradiated target or nontarget lesions selected under the modified itRECIST assessment. One and up to 3 lesions may be irradiated. At least 1 lesion must be T-I, while the remaining 2 may be T-I or NT-I. One to 3 measurable lesions are designated as T-I and are used to evaluate the irradiated lesion response. One to 5 measurable lesions are designated as T-NI. The irradiated response is based entirely on T-I lesions aggregating the sum of the largest diameter for all of the irradiated target lesions, while the nonirradiated response is based entirely on T-NI lesions aggregating the sum of the largest diameter for all of the nonirradiated target lesions. Overall target lesion response, nontarget lesion response, and new lesion appearance are defined as they are for RECIST v.1.1 and combined similarly to determine the itRECIST overall response for each visit. The overall response should include all lesions classified as target at baseline (sum of diameters of T-I and T-NI combined vs sum of diameters at baseline and at nadir), all nontarget lesions (NT-I and NT-NI) combined (classified as absent, present, or collectively showing unequivocal progression), and new lesions.

9.4.14.1.2 Treatment Beyond Progression

RECIST v.1.1 criteria and not modified itRECIST criteria will be used to evaluate considerations for treatment beyond progression. When assessing response, special consideration should be given to the tumor response characteristics associated with immunotherapy:

1. Measurable tumor size reduction may take longer with immunotherapy than with a cytotoxic regimen.
2. Response to immunotherapy may occur after appearance of PD, as assessed per conventional RECIST v.1.1 (see Appendix H). In particular, small, new lesions may appear in the presence of other responsive target lesions (pseudo-progression).
3. Durable stable disease (SD) may represent antitumor activity of immunotherapy.

Therefore, allowances to RECIST v.1.1 will be implemented in this study as described below.

Accumulating data indicate it is possible that some patients treated with immunotherapy may derive clinical benefit beyond initial RECIST-defined PD. The protocol therefore accommodates an option to keep patients on treatment beyond such initial progression if specific criteria are met.

Accordingly, patients will be permitted to continue treatment beyond initial RECIST v.1.1-defined PD if the following criteria are met:

1. Investigator-assessed overall clinical benefit from continued treatment with pembrolizumab and/or TAK-676. The assessment of clinical benefit should consider whether the patient is clinically deteriorating and unlikely to receive further benefit from continued treatment.
2. Tolerance of study drug(s).
3. Stable performance status.
4. Treatment beyond apparent progression will not delay an imminent intervention to prevent serious complications of PD (eg, central nervous system metastases).
5. Patient-provided written informed consent describing any reasonably foreseeable risks or discomforts, or other alternative treatment options.

The decision to continue treatment beyond initial RECIST v.1.1-defined progression should be discussed with the Takeda medical monitor and documented in the study records. Then, the patient may remain on the study and continue to receive monitoring according to the protocol-defined SOEs. Treatment should be discontinued permanently upon documentation of further PD. If the patient experiences rapid clinical deterioration as perceived by the investigator within 8 weeks of original PD and before additional radiographic assessment, the investigator can discontinue the study treatment without objective evidence of disease progression and report it as "symptomatic deterioration."

Patients who continue study therapy beyond initial RECIST v.1.1-defined progression will be considered to have investigator-assessed PD at the time of the initial progression event, if the progression is confirmed on the subsequent scan as detailed in SOE (Appendix A).

9.4.15 Biomarker, Pharmacodynamic, and PK Samples

9.4.15.1 Primary Specimen Collection

Blood samples will be collected via venipuncture or indwelling catheter at the time points detailed in Appendix A for plasma concentration measurements of TAK-676 (as applicable) and biomarker assessments (except for tumor biopsy). These samples must be collected on their own dedicated line, separate from the TAK-676/pembrolizumab administration line. The primary specimen collection is presented in Table 9.c.

If necessary, plasma samples collected for PK assessments may also be used for exploration of pharmacodynamic biomarkers. These plasma PK samples may only be used for this purpose after the final PK analysis has been completed.

Details on sample handling, storage, shipment, and analysis are provided in the Laboratory Manual.

Table 9.c Primary Specimen Collection

Specimen Name in Schedule of Procedures	Primary Specimen	Primary Specimen Derivative 1	Primary Specimen Derivative 2	Description of Intended Use	Sample Collection
Archival (banked) tumor tissue sample	FFPE block/slides	DNA RNA	Protein	Biomarker measurements	Required, if sample is available
Fresh tumor tissue biopsy sample	Fresh tumor tissue	FFPE block/slides	DNA/RNA/Protein	Biomarker measurements	Mandatory [a]
Plasma sample for circulating biomarkers	Plasma	Proteins	DNA/RNA/Metabolites	Biomarker measurements	Mandatory
Blood sample for immunophenotyping	Blood	Cells		Biomarker measurements	Mandatory
Blood sample for RNA	Blood	RNA		Biomarker measurements	Mandatory
Blood sample for receptor sequencing	Blood	DNA		Biomarker measurements	Mandatory
Plasma sample for cell-free DNA	Plasma	DNA		Biomarker measurements	Mandatory
Buccal epithelial cells sample for DNA	Tissue	DNA		Biomarker measurements	Mandatory
Blood sample for single cell RNA seq	Blood	RNA		Biomarker measurements	Mandatory [b]
Plasma sample for TAK-676 PK	Plasma			PK measurements	Mandatory

FFPE: formalin-fixed, paraffin-embedded; PK: pharmacokinetic(s).
[a] Tumor biopsy is mandatory only for patients receiving dose levels of TAK-676 that have previously shown pharmacodynamic activity. For patients receiving lower doses of TAK-676, tumor biopsies are optional but highly desired. Patients with a tumor biopsy obtained less than or equal to 3 months before initiation of TAK-676 (study Day 1), will not be required to undergo a repeat pretreatment biopsy, assuming 1) adequate tumor tissue is available for analysis (FFPE block preferred) and 2) no additional antineoplastic agents were administered since the time of the biopsy.
[b] To be collected from patients from select centers.

9.4.15.2 Tumor Biopsies Measurements

9.4.15.2.1 Banked Tumor

Banked formalin fixed paraffin-embedded tumor tissue or a minimum number of unstained slides (refer to Laboratory Manual for information on number of slides to be obtained) of the tumor tissue (ie, tumor tissue obtained at the time of the patient's original diagnosis and/or at the time of subsequent procedures conducted as part of the patient's standard care) will be collected, if available, from all enrolled patients to assess baseline features such as gene mutations, gene signatures, tumor mutation burden, immune cell content, or biomarkers of response or resistance

to treatment that may emerge from future nonclinical or clinical studies. See the Laboratory Manual for details.

9.4.15.2.2 Fresh Paired Tumor Biopsy

All patients with a safely accessible lesion outside the radiation field who enroll at dose levels of TAK-676 that have been shown to have pharmacodynamic activity will have mandatory tumor biopsy performed at screening and during Cycle 1 between Days 15 and 21 if, in the opinion of the investigator, such a biopsy would not place the patient at an unjustifiable risk. Pharmacodynamic activity is defined as evidence of TAK-676–mediated stimulation of the innate and/or adaptive immune system in the blood (refer to Section 9.4.15.1) and/or clinical response (CR/PR) in at least 1 patient from ongoing clinical studies. Dosing cohorts may be backfilled, as needed, to achieve this objective. Once pharmacodynamic activity is observed, biopsies will be required from all patients with safely accessible nonirradiated lesions enrolled in dose escalation. The number of patients projected to be needed to support a statistically supported posterior probability is outlined in Appendix K. Tumor biopsies are optional, but strongly encouraged, for patients enrolling at lower dose levels.

The tumor tissue biopsy at screening should be performed at least 2 days after the last dose of any prior antineoplastic therapy and within 3 weeks before the first dose of radiation. Screening biopsy should be performed only when the patient is considered otherwise eligible. The paired tumor biopsies should be performed on a lesion not targeted for irradiation and, when possible, collected from the same lesion (pre- and post-treatment). Patients with a tumor biopsy obtained less than or equal to 3 months before initiation of radiation therapy on trial will not be required to undergo a repeat pretreatment biopsy, assuming 1) adequate tumor tissue is available for analysis (formalin-fixed, paraffin-embedded block preferred) and 2) no additional antineoplastic agents were administered since the time of the biopsy.

Tumor biopsy procedures must be performed according to standard site practice. The accessible lesion biopsied should not have been previously irradiated. The tumor biopsy procedure will be performed by core needle, under radiological guidance if indicated, or surgically if the site of disease is superficial and palpable or visible. For tumor biopsies, at least 3 tissue cores of 1 cm each obtained using an 18-gauge end-cut needle are requested. These paired biopsies will be used to evaluate changes in biomarkers following treatment with radiation and pembrolizumab in combination with TAK-676, including the modulation of the tumor immune cell profile and impact on the tumor. The specific anatomical location of the biopsy should be noted upon collection.

9.4.15.3 Biomarker and Pharmacodynamic Measurements

In this study, several biomarkers and pharmacodynamic measurements will be assessed to test for correlation with PK, safety and, if possible, with efficacy. These biomarkers will be used to evaluate pharmacodynamic activity and patients who have a higher probability of response or of adverse reactions to radiation and pembrolizumab in combination with TAK-676. Because new

techniques continue to be developed, the methods and laboratories that will be recommended for the biomarker analyses are not specified.

The biomarker and pharmacodynamic specimen collection time points are displayed in Appendix A. Details regarding the preparation, handling, and shipping of samples are provided in the Laboratory Manual.

The following biomarker and pharmacodynamic measures will be tested:

Plasma Sample for Circulating Biomarkers

Plasma samples will be collected to monitor the treatment-induced changes in peripheral biomarkers, including cytokines and chemokines (for example IP-10, an IFN-inducible chemokine). The changes in cytokines and chemokines will be used to identify potential pharmacodynamic activity. Additional analysis may explore whether levels of any of these biomarkers correlate with probability of response or of adverse reactions to radiation and pembrolizumab in combination with TAK-676.

Blood Sample for Immunophenotyping

Blood samples will be collected for assessment of immunophenotypic changes in circulating immune cells induced by radiation and pembrolizumab in combination with TAK-676. These blood samples will be analyzed for the presence and changes in immune cell state by immunophenotyping; including, but not limited to B and T lymphocytes, monocytes, NK cells, and DCs.

Blood Sample for RNA

Blood samples for RNA will be collected for sequencing or expression analysis to monitor gene expression changes as a pharmacodynamic effect of radiation and pembrolizumab in combination with TAK-676 where acceptable by law. The induction of the STING agonism/type I IFN gene signature will be evaluated as part of this analysis. Additional blood samples collected at select centers may be analyzed by single cell RNA sequencing to determine changes in gene expression within particular cell types of interest, including T cells, NK cells, and monocytes.

Blood Sample for Receptor Sequencing

To monitor for induction of an adaptive immune response, blood samples will be collected to sequence T cell and B cell receptors where acceptable by law. Clonality and diversity of the receptors may be evaluated with any significant changes being associated with an expanded or contracted cellular population.

Plasma Sample for Cell-Free DNA

Plasma samples will be collected for evaluation of cell-free DNA where acceptable by law. From the cell-free DNA, circulating tumor DNA may be evaluated to assess tumor mutations.

9.4.15.4 PK Measurements

Details regarding the preparation, handling, and shipping of the PK samples are provided in the Laboratory Manual. Plasma samples for PK will be collected at the time points specified in Appendix A. The peripheral IV line on which PK/pharmacodynamics samples are to be drawn should be a dedicated line, separate from the TAK-676/pembrolizumab administration line. Ideally, the dedicated line should be contralateral, or at a minimum, distal to where TAK-676/pembrolizumab is being infused.

The timing, but not the total number of plasma samples may be modified during the study based on emerging PK data if a change in sampling scheme is considered necessary to better characterize the PK and pharmacodynamic relationship of TAK-676. A protocol amendment is not necessary for such modifications.

Plasma collected in this study will be used to measure the level of TAK-676. The plasma from selected patients may be used for exploratory analysis of TAK-676–related metabolites to understand TAK-676 metabolism and excretion mechanisms. The results of such exploratory metabolite analyses (qualitative or semiquantitative), if conducted, will be presented in a separate report and not in the CSR for this study.

9.4.15.5 DNA Analysis

As detailed in the SOE (Appendix A), a buccal epithelial cell sample will be collected during screening to extract DNA as a control sample for DNA mutation analysis from banked tumor and/or fresh tumor biopsies. In addition, the extracted DNA from such samples may be used in eventual pharmacogenomic evaluation to study the impact of genetic polymorphism, including drug metabolizing enzymes and/or transporters that may be implicated in the disposition of TAK-676, and polymorphisms in the gene encoding STING protein (*TMEM173*). The data resulting from such analyses, if performed, may be pooled with similar data from other TAK-676 clinical studies and will be reported separately and not within the CSR for this study. This sample may be collected during the study if the sample cannot be collected at screening.

9.5 Documentation of Patient Screen Failure

Investigators must account for all patients who sign informed consent.

If the patient is found to be not eligible before the first dose, the investigator should complete the applicable eCRF.

The primary reason for patient screen failure is recorded in the eCRF using the following categories:

- Death.
- AE.
- Failed inclusion criteria or did meet exclusion criteria.
- Protocol deviation.

- Lost to follow-up.
- Withdrawal of consent by patient.
- Study terminated by sponsor.

Patient identification codes assigned to patients who fail screening should not be reused. Patients may be allowed to rescreen within 28 days of initial screening following discussion between investigator and sponsor.

9.6 Completion of Study Treatment (for Individual Patients)

Patients will be considered to have completed study treatment if they discontinue pembrolizumab and/or TAK-676 for any of the reasons outlined in Section 9.7.

9.7 Discontinuation of Treatment With Study Drug and Patient Replacement

Patients will be informed that they have the right to discontinue study treatment at any time for any reason, without prejudice to their medical care. A patient's participation in the study may also be discontinued at any time at the discretion of the Investigator. It is possible that 1 study drug is permanently discontinued due to treatment-related AEs, while the patient continues to receive the other study drug and remains on treatment. Discontinuation of treatment occurs only when both study drugs are required to be discontinued due to AEs or upon the occurrence of any of the other non-AE criteria.

Treatment with radiation and/or study drugs may be discontinued for any of the following reasons:

- AE
- Protocol deviation (after discussion with sponsor).
- PD (tumor progression documented as assessed per RECIST v.1.1): as noted previously, patients who meet the criteria for PD per RECIST v.1.1 may continue to stay on study if they are clinically benefitting (see Section 9.4.14.1). In these patients, treatment discontinuation should occur when a subsequent scan confirms the initial PD, unless there are earlier clear signs of rapid clinical progression (clinical progression is defined as no imaging test performed to assess tumor status, but the investigator considers that patient decline is caused by tumor progression).
- Symptomatic deterioration (the patient presents a decline in health that recommends terminating treatment, and tumor imaging is performed and does not qualify for PD).
- Initiation of another systemic anticancer treatment.
- Pregnancy (patient must be discontinued).
- Study terminated by sponsor.
- Withdrawal of consent by patient.

- Lost to follow-up.
- Other (after discussion with sponsor).

Once both study drugs have been discontinued, all study procedures outlined for the EOT visit will be completed as specified in the SOE (Appendix A). If a patient is not able to return for the EOT visit, the EOT assessments may be performed at the time of treatment discontinuation. The primary reason for study drug discontinuation will be recorded on the eCRF. If the reason for ending treatment is also the reason for end of study (EOS) (for example, death), the EOS eCRF should be completed.

Patients not receiving all required doses of radiation, TAK-676, and pembrolizumab through Cycle 1 for reasons other than DLTs will not be considered DLT evaluable but may remain on study. If a patient is DLT inevaluable, patient replacement may not be mandatory. In this case, the decision for patient replacement can be determined based on the number of DLT-evaluable patients in the cohort, as agreed between the sponsor and investigators.

In the case of study termination by the sponsor, eligible patients may have continued access to TAK-676/pembrolizumab as described in Section 6.3.5.

9.8 Posttreatment Follow-up Assessments

Some patients may discontinue study drug for reasons other than PD; these patients will remain in the study for follow-up and will be followed every 12 weeks (±1 week) from the EOT visit until the occurrence of PD, loss to follow-up, consent withdrawal, the start of subsequent systemic antineoplastic therapy, study termination, or death, whichever occurs first. Survival status, date of disease progression based on available local data, and subsequent anticancer therapies will be collected during this follow-up period. This information may be collected by methods that include, but are not limited to, telephone, email, mail, or retrieval from online or other databases (eg, Social Security indexes).

The EOS eCRF is to be completed when the patient discontinues from the follow-up period.

Note: Related SAEs must be reported to the Global Pharmacovigilance department or designee. This includes deaths or SAEs that the investigator considers related to study drug that occur during posttreatment follow-up. Refer to Section 10.0 for details regarding definitions, documentation, and reporting of SAEs.

9.9 Completion of Study (for Individual Patients)

Patients will be considered to have completed the study if they are discontinued from both study drugs and 1 or more of the following situations occur:

- Death.
- Consent withdrawal.
- Study terminated by the sponsor.

- Lost to follow-up.
- Completion of follow-up period and assessments, if applicable.
- PD.
- Start of new systemic anticancer treatment.
- Transfer of patient to a long-term safety study, single-patient investigational new drug application, or similar program.

The consequence of study withdrawal is that no new information will be collected from the withdrawn patient and added to the existing data or any database.

At this time, the EOS eCRF should be completed.

9.10 Study Compliance

Radiation treatment and study drug(s)will be administered or dispensed only to eligible patients under the supervision of the investigator or identified subinvestigator(s). The appropriate study personnel will maintain records of study drug receipt and dispensing.

Tests and procedures should be performed on schedule; however, unless otherwise specified, occasional changes are allowable within a 3-day window for holidays, vacations, and other administrative reasons. If extenuating circumstances prevent a patient from beginning treatment or completing a scheduled procedure or assessment within this time, the patient may continue the study only with the written permission of the medical monitor. If an AE occurs after study radiation treatment (irrespective of causality) that leads to a delay in the start of planned study drug(s) administration on Cycle 1 Day 1, Cycle 1 Day 1 may be delayed for up to 1 week following discussion with the Medical Monitor or designee. Any Cycle 1 Day 1 delays beyond this timeframe would require additional approval from the Medical Monitor or designee.

Allowable windows on dosing days are described in Section 8.1.3.

If a dose of TAK-676 is held for up to 21 days for reasons unrelated to toxicity, the patient may be discontinued from the study following a discussion between the investigator and the sponsor.

10.0 ADVERSE EVENTS

10.1 Definitions

10.1.1 Pretreatment Event Definition

A pretreatment event is any untoward medical occurrence in a patient or subject who has signed informed consent to participate in a study but before administration of any study medication; it does not necessarily have to have a causal relationship with study participation.

10.1.2 AE Definition

AE means any untoward medical occurrence in a patient administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not it is related to the medicinal product. This includes any newly occurring event or a previous condition that has increased in severity or frequency since the administration of study drug.

All abnormal laboratory values will be reviewed by the investigator but only those abnormal values that lead to discontinuation or delay in treatment, dose modification, therapeutic intervention, or are considered by the investigator to be a clinically significant change from baseline will be assessed as AEs.

10.1.3 SAE Definition

SAE means any untoward medical occurrence that at any dose:

- Results in **death.**
- Is **life-threatening** (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient **hospitalization or prolongation of an existing hospitalization** (see clarification in the paragraph in Section 10.2 on planned hospitalizations).
- Results in **persistent or significant disability or incapacity**. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- Is a congenital anomaly/birth defect.
- Is a **medically important event**. This refers to an AE that may not result in death, be immediately life-threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, it may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (eg, prion protein transmitting transmissible spongiform encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent. In addition to the above examples, any event that the investigator considers to be medically important will be considered an SAE.

In this study, intensity for each AE, including any laboratory abnormality, will be determined using the NCI CTCAE, Version 5.0. Clarification should be made between an SAE and an AE that is considered severe in intensity (Grade 3 or 4) because the terms *serious* and *severe* are NOT

synonymous. The general term *severe* is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000/mm^3 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

10.2 Procedures for Recording and Reporting AEs and SAEs

All AEs spontaneously reported by the patient or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded on the appropriate page of the eCRF (see Section 10.3 for the period of observation). Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as a single comprehensive event.

Regardless of causality, SAEs (as defined in Section 10.1.3) must be reported (see Section 10.3 for the period of observation) by the investigator to the Takeda Global Pharmacovigilance department or designee within 24 hours of becoming aware of the event. This should be done by transmitting an electronic data capture (EDC) SAE report. If transmission of an EDC SAE report is not feasible within 24 hours of becoming aware of this event, then a Takeda paper-based SAE form should be submitted via fax at the fax numbers provided below. The SAE form, created specifically by Takeda, will be provided to each clinical study site. A sample of the paper-based SAE form and processing directions are found in the Study Manual. Information in the SAE report or form must be consistent with the data provided on the eCRF.

In case of faxing a paper-based SAE form, site personnel need to confirm successful transmission of all pages and include an email address on the fax cover sheet so that an acknowledgment of receipt can be returned via email within 1 business day.

Fax Numbers:

- United States and Canada: + 1-800-963-6290
- Rest of World: + 1-202-315-3560

Email submission of SAE forms with a PDF attachment should only be used in the case where fax is not possible, and EDC is not feasible within 24 hours of receiving the event. In case of email, site personnel need to confirm successful transmission by awaiting an acknowledgment of the receipt via email within 1 business day.

Email Address:

- takedaoncocases@cognizant.com

If SAEs are reported via fax or by email, EDC/RAVE must be updated as soon as possible with the appropriate information.

If information not available at the time of the first report becomes available at a later date, then the investigator will transmit a follow-up EDC SAE report (or a paper-based SAE form if an EDC SAE report is not feasible) or provide other documentation immediately within 24 hours of receipt. Copies of any relevant data from the hospital notes (eg, ECGs, laboratory tests, discharge summary, postmortem results) should be sent to the addressee, if requested.

All SAEs should be followed up until resolution or permanent outcome of the event. The timelines and procedure for follow-up reports are the same as those for the initial report.

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the study are not to be considered AEs unless the condition deteriorated in an unexpected manner during the study; eg, surgery was performed earlier or later than planned.

For both serious and nonserious AEs, the investigator must determine both the severity (toxicity grade) of the event and the relationship of the event to study drug administration.

Severity (toxicity grade) for each AE, including any laboratory abnormality, will be determined using the NCI CTCAE, Version 5.0. The criteria are provided in the Study Manual.

Relationship of the event to study drug administration (ie, its causality) will be determined by the investigator responding yes (related) or no (unrelated) to this question: Is there a reasonable possibility that the AE is associated with the study drug/s?

10.3 Monitoring of AEs and Period of Observation

AEs, both nonserious and serious, will be monitored throughout the study as follows:

- All AEs will be reported from the time of signing of the informed consent through 30 days after the administration of the last dose of study drug(s) or before initiation of new anticancer therapy (whichever comes first) and recorded in the eCRFs.

  AEs that the investigator considers to be immune-mediated will be reported through 90 days after the administration of the last dose of study drug(s) or before initiation of new anticancer therapy (whichever comes first) and recorded in the eCRFs.

- SAEs will be reported to the Takeda Global Pharmacovigilance department or designee from the time of signing of informed consent through 30 days after administration of the last dose of study drug(s) or before initiation of new anticancer therapy (whichever comes first) and recorded in the eCRF.

  Only related SAEs occurring greater than 30 days after the last dose of study drug(s) (until patient completes the study or initiates new anticancer therapy) must be reported to the Takeda Global Pharmacovigilance department or designee.

SAEs will be monitored until they have resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

10.4 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug. The sponsor must also be contacted immediately by sending a completed pregnancy form to the Takeda Global Pharmacovigilance department or designee. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor must also be contacted immediately by sending a completed pregnancy form to the Takeda Global Pharmacovigilance department or designee. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

Pregnancies are to be reported through 120 days after the last dose of study drug.

10.5 Procedures for Reporting Product Complaints or Medication Errors (Including Overdose)

10.5.1 TAK-676

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately report this via the phone numbers or email addresses provided in the Study Manual.

A medication error is a preventable event that involves an identifiable patient and leads to inappropriate medication use, which may result in patient harm. Overdoses and underdoses constitute medication errors. Individuals who identify a potential medication error (including overdose) situation should immediately report this via the phone numbers or email addresses provided in the Study Manual.

Product complaints and medication errors in and of themselves are not AEs. If a product complaint or a medication error results in an SAE, the SAE should be reported.

10.5.2 Pembrolizumab

"Pembrolizumab Injection 100 mg/4 mL (25 mg/mL) solution in a single-dose vial" will be either labeled as study material and supplied by the sponsor or sourced locally by the clinical site when arrangements have been made and agreed to by sponsor and where regulations allow for clinical site sourcing, appropriate labeling, and compliance with local and regional regulations. Information on the formulation, packaging, and storage of pembrolizumab is provided in the most recent package insert. For product complaints related to pembrolizumab provided by the sponsor, please see the Pharmacy Manual. For product complaints related to pembrolizumab sourced

locally by the clinical site, utilize standard methods to contact product manufacturer or regulatory agency as per standard site process.

10.6 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities

The sponsor will be responsible for reporting all suspected unexpected serious adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities, investigators, and IRBs and IECs, as applicable, in accordance with national regulations. Relative to the first awareness of the event by/or further provision to the sponsor or sponsor's designee, SUSARs will be submitted to the regulatory authorities as expedited reports within 7 days for fatal and life-threatening events and within 15 days for other serious events, unless otherwise required by national regulations. The sponsor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the investigational medicinal product's administration or in the overall conduct of the study. The investigational site also will forward a copy of all expedited reports to his or her IRB or IEC in accordance with national regulations.

11.0 STUDY-SPECIFIC COMMITTEES

No steering committee, data safety monitoring committee, or clinical endpoint committee will be used in this study.

12.0 DATA HANDLING AND RECORDKEEPING

The full details of procedures for data handling will be documented in the data management plan. If selected for coding, AEs, medical history, and concurrent conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Drugs will be coded using the World Health Organization (WHO) Drug Dictionary.

12.1 eCRFs

Completed eCRFs are required for each patient who signs an ICF.

The sponsor or its designee will supply investigative sites with access to eCRFs and will make arrangements to train appropriate site staff in the use of the eCRF. These forms are used to transmit the information collected in the performance of this study to the sponsor, contract research organization (CRO) partners, and regulatory authorities. Investigative sites must complete eCRFs in English.

After completion of the entry process, computer logic checks will be run to identify items such as inconsistent dates, missing data, and questionable values. Queries may be issued by Takeda personnel (or designee) and will be answered by the site.

Any change of, modification of, or addition to the data on the eCRFs should be made by the investigator or appropriate site personnel. Corrections to eCRFs are recorded in an audit trail that captures the old information, the new information, identification of the person making the correction, the date the correction was made, and the reason for the change.

The principal investigator must review the eCRFs for completeness and accuracy and must sign and date the appropriate eCRFs as indicated. Furthermore, the principal investigator must retain full responsibility for the accuracy and authenticity of all data entered on the eCRFs.

eCRFs will be reviewed for completeness and acceptability at the study site during periodic visits by study monitors. The sponsor (or designee) will be permitted to review the patient's medical and hospital records pertinent to the study to ensure accuracy of the eCRFs. The completed eCRFs are the sole property of the sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate governmental health or regulatory authorities, without written permission of the sponsor.

12.2 Record Retention

The investigator agrees to keep the records stipulated in Section 12.1 and those documents that include (but are not limited to) the study-specific documents, the identification log of all participating patients, medical records, temporary media such as thermal-sensitive paper, source worksheets, all original signed and dated ICFs, patient authorization forms regarding the use of personal health information (if separate from the ICFs), electronic copies of eCRFs including the audit trail, and detailed records of drug disposition to enable evaluations or audits from regulatory authorities and the sponsor (or designees). Any source documentation printed on degradable thermal-sensitive paper should be photocopied by the site and filed with the original in the patient's chart to ensure long-term legibility. Furthermore, ICH E6 Section 4.9.5 requires the investigator to retain essential documents specified in ICH E6 (Section 8) until at least 2 years after the last approval of a marketing application for a specified drug indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory authorities are notified. In addition, ICH E6 Section 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the clinical study site agreement between the investigator and sponsor.

Refer to the clinical study site agreement for the sponsor's requirements for record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

13.0 STATISTICAL METHODS

13.1 Statistical and Analytical Plans

A statistical analysis plan will be prepared and finalized before database lock. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives.

13.1.1 Analysis Sets

The analysis sets will include the following:

- **Safety analysis set:** Patients who have received at least 1 dose of radiation will be included in all safety analyses.
- **PK analysis set:** Patients dosed with TAK-676 for whom plasma concentration data have been collected will be used for PK analyses.
- **DLT-evaluable analysis set:** The DLT-evaluable analysis set will include patients who receive all Cycle 1 doses of TAK-676 in addition to pembrolizumab and radiation without experiencing a DLT or who have a DLT during Cycle 1.

  Patients who receive only 1 or 2 doses of radiation will not be considered DLT evaluable and may or may not be replaced within the same cohort but will be allowed to continue on study.
- **Comprehensive response-evaluable analysis set**: The comprehensive response-evaluable analysis set, a subset of the safety analysis set including patients with measurable disease at baseline, at least 1 posttreatment evaluation or who discontinued before their first postbaseline tumor assessment will be used for analyses of response.

  - Response-evaluable analysis subset: The response-evaluable analysis subset, a subset of the safety analysis and comprehensive response evaluable analysis set including patients with measurable disease at baseline and at least 1 posttreatment evaluation, will be used as a subset analysis of response.
- **Pharmacodynamic analysis set**: The pharmacodynamic analysis set will include those patients in the safety population who have received at least 1 dose of TAK-676 and who have baseline and at least 1 postbaseline pharmacodynamic sample assessment.

13.1.2 Analysis of Demographics and Other Baseline Characteristics

Patient demographic and baseline characteristics will be summarized descriptively. Variables to be analyzed include sex, age, race, medical history, prior medications/therapies, ECG findings and other parameters as appropriate. For continuous variables, descriptive statistics (number, mean, standard deviation, median, minimum, and maximum) will be provided. For categorical variables, patient counts and percentages will be provided. Categories for missing data will be presented as needed.

13.1.3 Efficacy Analysis

Efficacy is not the primary endpoint for this study. Secondary efficacy endpoints include ORR, ORRnonirradiated, ORRirradiated, DOR, DORnonirradiated, DORirradiated, TTR, TTRnonirradiated, and TTRirradiated. No formal statistical tests will be performed for these secondary endpoints. Response assessments by investigator are based on RECIST v.1.1 (Eisenhauer et al. 2009) and a modified itRECIST (where intratumoral therapy references are largely replaced with radiation therapy) referenced in Section 9.4.14.

ORR is defined as the proportion of patients who achieve cCR or cPR (determined by the investigator) during the study in response-evaluable population.

ORRnonirradiated is defined as the proportion of patients who achieve cCR or cPR (determined by the investigator) in the tumor lesions lying outside of the radiation field during the study in response-evaluable population.

ORRirradiated is defined as the proportion of patients who achieve cCR or cPR (determined by the investigator) in the tumor lesions lying within the radiation field during the study in response-evaluable population.

DOR is the time from the date of first documentation of a cPR or better to the date of first documentation of PD for responders (cPR or better). Responders without documentation of PD will be censored at the date of last response assessment that is SD or better.

DORnonirradiated is the time from the date of first documentation of a cPR or better in the tumor lesions lying outside of the radiation field to the date of first documentation of nonirradiated PD in those lesions for nonirradiated responders (cPR or better). Nonirradiated responders without documentation of nonirradiated PD will be censored at the date of last response assessment that is nonirradiated SD or better.

DORirradiated is the time from the date of first documentation of a cPR or better in the tumor lesions lying within the radiation field to the date of first documentation of irradiated PD in those lesions for local responders (cPR or better). Local responders without documentation of local PD will be censored at the date of last response assessment that is local SD or better.

TTR is defined as the time from the date of first dose administration to the date of first documented cPR or better by the investigator.

TTRnonirradiated is defined as the time from the date of first dose administration to the date of first documented cPR or better in the tumor lesions lying outside of the radiation field by the investigator.

TTRirradiated is defined as the time from the date of first dose administration to the date of first documented cPR or better in the tumor lesions lying within the radiation field by the investigator.

ORR, ORRnonirradiated, and ORRirradiated will be summarized using descriptive statistics with 95% CI. DOR, DORnonirradiated, DORirradiated, TTR, TTRirradiated, and TTRirradiated will also be analyzed using Kaplan-Meier method for response-evaluable analysis set.

13.1.4 Pharmacokinetic Analysis

13.1.4.1 PK Analysis

Individual TAK-676 concentration-time data will be presented in listings and tabulated using summary statistics by dose cohort. Individual and mean concentration-time profiles will be plotted by dose cohort.

13.1.4.2 PK Sampling Intended for Population PK Analysis

The PK data collected in this study are intended to contribute to future population PK analyses of TAK-676. These population PK analyses may additionally include data collected in other TAK-676 clinical studies. The plan for the population PK analysis will be defined separately and the results reported separately.

13.1.5 Pharmacodynamic Analysis

Changes in T-cell infiltration levels between the pretreatment and on-treatment tumor biopsies will be summarized.

Changes between pretreatment and on-treatment peripheral blood samples in levels of plasma biomarkers, including cytokines and chemokines, and gene expression, including the STING agonism/type I IFN signature may be summarized.

Relationship between response to combination treatment regimen and mutations or polymorphisms in immune response or DNA damage repair genes may be analyzed. Relationship between response to combination treatment regimen and polymorphisms in the STING gene (*TMEM173*) and drug transporter genes relevant to TAK-676 may be investigated.

13.1.6 PK/Pharmacodynamic Analysis

The relationship between TAK-676 plasma exposure and pharmacodynamic response (eg, degree of immune activation, changes in cytokines/chemokines, gene expression changes) will be explored on an ongoing basis as PK and pharmacodynamic data become available to understand the PK/pharmacodynamic relationship of TAK-676. Data permitting, mathematical models may be used to describe this relationship, and such models may be used to predict the dose/schedule of TAK-676 that provides the desired exposure and pharmacological response for future evaluation. These data may be presented graphically as well as summarized in the CSR. The analysis will be performed in the pharmacodynamic population.

In addition, the PK/pharmacodynamic data collected in the study during dose escalation may be used to inform the quantitative systems pharmacology model that may be used to further refine the dose/schedule for TAK-676. Furthermore, the PK/pharmacodynamic data collected in this study may be pooled with similar data from other clinical studies for population analysis purposes. The results of such PK/pharmacodynamic and population PK/pharmacodynamic analyses and quantitative systems pharmacology modeling may not be presented in the CSR for this study but will be presented in a separate report.

13.1.7 ECG Analysis

A summary of ECG abnormalities will be presented by visit. ECG parameters (QT, QTcF, PR interval, QRS, and heart rate) will be summarized at each scheduled time point, along with mean change from baseline to each posttreatment time point.

13.1.8 Safety Analysis

Safety will be evaluated by the frequency of AEs, severity and types of AEs, and by changes from baseline in patients' vital signs, weight, and clinical laboratory results using the safety analysis set.

Exposure to study drug and reasons for discontinuation will be tabulated.

TEAEs that occur after administration of the first dose of study and through 30 days after the last dose of study drug will be tabulated.

Related immune-mediated AEs as determined by the investigator that occur after administration of the first dose of study drug and through 90 days after administration of the last dose of study drug or before initiation of new anticancer therapy (whichever comes first) will be tabulated.

AEs will be tabulated according to the MedDRA and will include the following categories:

- TEAEs.
- Drug-related TEAEs.
- Grade 3 or higher TEAEs.
- Grade 3 or higher drug-related TEAEs.
- The most commonly reported TEAEs (ie, those reported by ≥10% of all patients).
- TESAEs (related and regardless of relationship).
- TEAEs leading to study drug modification and discontinuation.

The incidence of DLTs will be tabulated using the DLT-evaluable analysis set.

Descriptive statistics for the actual values of clinical laboratory parameters (and/or change from baseline in clinical laboratory parameters) will be presented for all scheduled measurements over time. Mean laboratory values over time will be plotted for key laboratory parameters.

Descriptive statistics for the actual values (and/or the changes from baseline) of vital signs and weight will be tabulated by scheduled time point. ECOG performance scores will be summarized using a shift table.

Shift tables for laboratory parameters will be generated for changes in NCI CTCAE grade from baseline to worst postbaseline value. Graphical displays of key safety parameters, such as scatter plots of baseline vs worst postbaseline values, may be used to understand the study drugs' safety profile.

All concomitant medications collected from the first dose of study drug throughout the study period will be classified to Preferred Terms according to the WHO Drug Dictionary.

Additional safety analyses may be performed to most clearly enumerate rates of toxicities and to further define the safety profile of study drugs.

13.2 Interim Analysis and Criteria for Early Termination

Although no formal interim analysis is planned, investigators and sponsor representatives will review accruing data to determine dose escalation decision and number of patients per cohort in the dose escalation phase.

13.3 Determination of Sample Size

Approximately 65 patients with metastatic NSCLC, TNBC, or SCCHN will be enrolled in this study, to achieve a maximum of 55 DLT evaluable patients. They will receive pembrolizumab administered at 200 mg IV every 3 weeks, and TAK-676 administered in a dose escalating fashion followed by BOIN design, with an explorable dose range of 0.2 to 9.0 mg administered on Days 1, 8, and 15 of every 21-day cycle.

The details of dose escalation rules are specified in Section 8.3.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Study-Site Monitoring Visits

Monitoring visits will be conducted periodically during the study to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data recorded on the eCRFs. Source documents are defined as original documents, data, and records. The investigator and institution guarantee access to source documents by the sponsor or its designee (CRO) and by the IRB or IEC.

In the event a monitor cannot visit the site in a timely manner due to the COVID-19 pandemic, alternative monitoring approaches such as remote source data verification or telephone contact may be used to ensure data quality and integrity and maintain patient safety. Alternative monitoring approaches should be used only where allowed by the local Health Authority and permitted by the IRB/IEC.

All aspects of the study and its documentation will be subject to review by the sponsor or designee including, but not limited to, the investigator's binder, study medication, subject medical records, informed consent documentation, documentation of subject authorization to use personal health information (if separate from the ICFs), and review of eCRFs and associated source documents. It is important that the investigator and other study personnel are available during the monitoring visits and that sufficient time is devoted to the process.

14.2 Protocol Deviations

The investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study subjects. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the investigator should consult with the sponsor or designee (and IRB or IEC, as required) to determine the appropriate course of action. There will be no exemptions (a prospectively approved deviation) from the inclusion or exclusion criteria.

The site should document all protocol deviations in the subject's source documents. In the event of a significant deviation, the site should notify the sponsor or its designee (and IRB or EC, as required). Significant deviations include, but are not limited to, those that involve fraud or misconduct, increase the health risk to the subject, or confound interpretation of the primary study assessment.

The sponsor will assess any protocol deviation; if it is likely to affect to a significant degree the safety and rights of a subject or the reliability and robustness of the data generated, it may be reported to regulatory authorities as a serious breach of GCP and the protocol.

14.3 Quality Assurance Audits and Regulatory Agency Inspections

The study site also may be subject to quality assurance audits by the sponsor or designees. In this circumstance, the sponsor-designated auditor will contact the site in advance to arrange an auditing visit. The auditor may ask to visit the facilities where laboratory samples are collected, where the medication is stored and prepared, and any other facility used during the study. In addition, there is the possibility that this study may be inspected by regulatory agencies, including those of foreign governments (eg, the US FDA, the United Kingdom [UK] Medicines and Healthcare products Regulatory Agency [MHRA], the Pharmaceuticals and Medical Devices Agency of Japan [PMDA]). If the study site is contacted for an inspection by a regulatory body, the sponsor should be notified immediately. The investigator and institution guarantee access for quality assurance auditors to all study documents as described in Section 14.1.

15.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (ie, patients) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the ICH Harmonised Tripartite Guideline for GCP. Each investigator will conduct the study according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the responsibilities of the investigator that are listed in Appendix B. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

15.1 IRB and/or IEC Approval

IRBs and IECs must be constituted according to the applicable requirements of each participating region. The sponsor or designee will require documentation noting all names and titles of members who make up the respective IRB or IEC. If any member of the IRB or IEC has direct participation in this study, written notification regarding his or her abstinence from voting must also be obtained. Those American sites unwilling to provide names and titles of all members because of privacy and conflict of interest concerns should instead provide a Federal wide Assurance number or comparable number assigned by the US Department of Health and Human Services.

The sponsor or designee will supply relevant documents for submission to the respective IRB or IEC for the protocol's review and approval. This protocol, the IB, a copy of the ICF, and, if applicable, subject recruitment materials and advertisements and other documents required by all

applicable laws and regulations must be submitted to a central or local IRB or IEC for approval. The IRB's or IEC's written approval of the protocol and subject informed consent must be obtained and submitted to the sponsor or designee before commencement of the study, ie, before shipment of the sponsor-supplied drug or study-specific screening activity. The IRB or IEC approval must refer to the study by its exact protocol title, number, and version date; identify versions of other documents (eg, ICF) reviewed; and state the approval date. If required by country or regional regulations or procedures, approval from the competent regulatory authority will be obtained before commencement of the study or implementation of a substantial amendment. The sponsor will notify site once the sponsor has confirmed the adequacy of site regulatory documentation and, when applicable, the sponsor has received permission from the competent authority to begin the study. Until the site receives notification, no protocol activities, including screening, may occur.

Sites must adhere to all requirements stipulated by their respective IRB or IEC. This may include notification to the IRB or IEC regarding protocol amendments, updates to the ICF, recruitment materials intended for viewing by subjects, local safety reporting requirements, reports and updates regarding the ongoing review of the study at intervals specified by the respective IRB or IEC, and submission of the investigator's final status report to IRB or IEC. All IRB and IEC approvals and relevant documentation for these items must be provided to the sponsor (or designee).

Patients incentives should not exert undue influence for participation. Payments to subjects must be approved by the IRB or IEC and sponsor.

15.2 Patient Information, Informed Consent, and Patient Authorization

Written consent documents will embody the elements of informed consent as described in the Declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all applicable laws and regulations. The ICF describes the planned and permitted uses, transfers, and disclosures of the patient's personal and personal health information for purposes of conducting the study. The ICF and the patient information sheet (if applicable) further explain the nature of the study, its objectives, and potential risks and benefits, and the date informed consent is given. The ICF will detail the requirements of the participant and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care.

The investigator is responsible for the preparation, content, and IRB or IEC approval of the ICF and, if applicable, the patient authorization form. The ICF, patient authorization form (if applicable), and patient information sheet (if applicable) must be approved by both the IRB or IEC and the sponsor before use.

The ICF, patient authorization form (if applicable), and patient information sheet (if applicable) must be written in a language fully comprehensible to the prospective patient. It is the responsibility of the investigator to explain the detailed elements of the ICF, patient authorization form (if applicable), and patient information sheet (if applicable) to the patient. Information should be given in both oral and written form whenever possible and in the manner deemed appropriate by

the IRB or IEC. If the patient is not capable of rendering adequate written informed consent, then the patient's legally acceptable representative may provide such consent for the patient in accordance with applicable laws and regulations.

The patient, or the patient's legally acceptable representative (if acceptable at site), must be given ample opportunity to (1) inquire about details of the study and (2) decide whether to participate in the study. If the patient, or the patient's legally acceptable representative, determines that he or she will participate in the study, then the ICF and patient authorization form (if applicable) must be signed and dated by the patient, or the patient's legally acceptable representative, at the time of consent and before the patient enters into the study. The patient or the patient's legally acceptable representative should be instructed to sign using their legal names, not nicknames, using a ballpoint pen with either blue or black ink. The investigator must also sign and date the ICF and patient authorization (if applicable) at the time of consent and before the patient enters into the study; however, the sponsor may allow a designee of the investigator to sign to the extent permitted by applicable law.

Once signed, the original ICF, patient authorization form (if applicable), and patient information sheet (if applicable) will be stored in the investigator's site file. The investigator must document the date the patient signs the informed consent in the patient's medical record. Copies of the signed ICF, the signed patient authorization form (if applicable), and patient information sheet (if applicable) shall be given to the patient.

All revised ICFs must be reviewed and signed by relevant patients or the relevant patient's legally acceptable representative in the same manner as the original informed consent. The date the revised consent was obtained should be recorded in the patient's medical record, and the patient should receive a copy of the revised ICF.

15.3 Patient Confidentiality

The sponsor and designees affirm and uphold the principle of the patient's right to protection against invasion of privacy. Throughout this study, a patient's source data will be linked to the sponsor's clinical study database or documentation only via a unique identification number. As permitted by all applicable laws and regulations, limited patient attributes, such as sex, age, or date of birth, and patient initials may be used to verify the patient and accuracy of the patient's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires the investigator to permit its monitor or designee's monitor, representatives from any regulatory authority (eg, US FDA, UK MHRA, Japan PMDA), the sponsor's designated auditors, and the appropriate IRBs and IECs to review the patient's original medical records (source data or documents) including, but not limited to, laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a patient's study participation, and autopsy reports. Access to a patient's original medical records requires the specific authorization of the patient as part of the informed consent process (see Section 15.2).

Copies of any patient source documents that are provided to the sponsor must have certain identifying personal information removed, eg, patient name, address, and other identifier fields not collected on the patient's eCRF.

15.4 Publication, Disclosure, and Clinical Trial Registration Policy

15.4.1 Publication

The investigator is obliged to provide the sponsor with complete test results and all data derived by the investigator from the study. During and after the study, only the sponsor may make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the clinical study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and advertisements, is the sole responsibility of the sponsor.

The sponsor may publish any data and information from the study (including data and information generated by the investigator) without the consent of the investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the clinical study site agreement. In the event of any discrepancy between the protocol and the clinical study site agreement, the clinical study site agreement will prevail.

15.4.2 Clinical Trial Registration

To ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable laws, regulations, and guidance, Takeda will, at a minimum, register interventional clinical trials it sponsors anywhere in the world on ClinicalTrials.gov or other publicly accessible websites on or before start of study, as defined by Takeda policy/standards. Takeda contact information, along with investigator's city, state (for Americas investigators), country, and recruiting status will be registered and available for public viewing.

As needed, Takeda and investigator/site contact information may be made public to support participant access to trials via registries. In certain situations/registries, Takeda may assist participants or potential participants in finding a clinical trial by helping them locate trial sites closest to their homes by providing the investigator name, address, and phone number via email/phone or other methods preferred by callers requesting trial information. Once patients receive investigator contact information, they may call the site requesting enrollment into the trial. The investigative sites are encouraged to handle the trial inquiries according to their established patient screening process. If the caller asks additional questions beyond the topic of trial enrollment, they should be referred to the sponsor.

Any investigator who objects to Takeda providing this information to callers must provide Takeda with a written notice requesting that their information not be listed on the registry site.

15.4.3 Clinical Trial Results Disclosure

Takeda will post the results of clinical trials on ClinicalTrials.gov, and other publicly accessible websites (including the Takeda corporate site) and registries, as required by Takeda policy/standards, applicable laws, and/or regulations.

Data Sharing

The sponsor is committed to responsible sharing of clinical data with the goal of advancing medical science and improving patient care. Qualified independent researchers will be permitted to use data collected from patients during the study to conduct additional scientific research, which may be unrelated to the study drug or the patient's disease. The data provided to external researchers will not include information that identifies patients personally.

15.5 Insurance and Compensation for Injury

Each patient in the study must be insured in accordance with the regulations applicable to the site where the patient is participating. If a local underwriter is required, then the sponsor or sponsor's designee will obtain clinical study insurance against the risk of injury to clinical study patients. Refer to the clinical study site agreement regarding the sponsor's policy on patient compensation and treatment for injury. If the investigator has questions regarding this policy, he or she should contact the sponsor or sponsor's designee.

16.0 REFERENCES

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Appendix A Schedule of Events

Table 1 SOE for Treatment With Radiation Therapy + Pembrolizumab + TAK-676 (21-Day Cycle)

	Screening [a]	RT	Cycle 1				Cycle 2			Cycle 3			Cycle 4 and Beyond			EoT/ EoS	Follow Up [x]
		Day -8 to Day -2	Day 1	Day 8	Day 15	Day 15-21	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15		
Study Procedures																	
Informed consent	X																
Inclusion/exclusion criteria	X																
Demographics	X																
Medical history [b]	X																
Physical examination [b]	X		X	X	X		X			X			X			X	
Height	X																
Weight [c]	X		X	X	X		X	X	X	X	X	X	X	X	X	X	
Vital signs [d]	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	
ECOG performance status [e]	X	X	X				X			X			X			X	
Safety 12-lead ECG [f]	X		X				X			X			X			X	
Echocardiogram or MUGA	X																
Tumor assessment for solid tumors by RECIST v.1.1 (CT/MRI) [g]	X								X						X		X
Monitoring of concomitant medications and procedures		Recorded from the signing of ICF through 30 days after the last dose of study drug or before initiation of new anticancer therapy (whichever comes first).															
AE reporting		Recorded from the signing of ICF through 30 days after the last dose of study drug(s) or before initiation of new anticancer therapy (whichever comes first). AEs that the investigator considers immune-mediated will be recorded through 90 days after administration of the last dose of study drug(s) or before initiation of new anticancer therapy (whichever comes first).															

Table 1 SOE for Treatment With Radiation Therapy + Pembrolizumab + TAK-676 (21-Day Cycle)

	Screening [a]	RT	Cycle 1				Cycle 2			Cycle 3			Cycle 4 and Beyond			EoT/ EoS	Follow Up [x]
		Day -8 to Day -2	Day 1	Day 8	Day 15	Day 15-21	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15		
		SAEs will be reported from signing of the ICF through 30 days after the last dose of study drug(s) or before initiation of new anticancer therapy (whichever comes first). Only related SAEs occurring >30 days after the last dose of study drug(s) (until patient completes the study or initiates new anticancer therapy) must be reported.															
Radiation Administration																	
RT administration [h, i]		X															
Dosing																	
TAK-676 administration [j]			X	X	X		X	X	X	X	X	X	X	X	X		
Pembrolizumab administration [k]			X				X			X			X				
Samples/Laboratory Assessments																	
Pregnancy test [l]	X	X	X				X			X			X			X	
Hematology/ chemistry [l]	X		X	X	X		X	X	X	X	X	X	X			X	
Thyroid function tests [l, m]	X		X							X			X			X	
C-reactive protein [l]	X		X				X			X			X			X	
Creatine phosphokinase [l]	X		X	X	X		X	X	X	X	X	X	X			X	
Ferritin [l]	X		X	X	X		X	X	X	X	X	X	X			X	
Coagulation	X		X				X			X			X			X	
Urinalysis [n]	X		X				X			X			X			X	
Fresh tumor tissue biopsy [o]	X					X											
Plasma sample for circulating biomarkers [p]	X		X3		X3		X			X			X (Cycles 4, 9, 15 only)				
Blood sample for	X		X2		X2		X			X			X (Cycles 4, 9, 15				

Table 1 SOE for Treatment With Radiation Therapy + Pembrolizumab + TAK-676 (21-Day Cycle)

	Screening [a]	RT	Cycle 1				Cycle 2			Cycle 3			Cycle 4 and Beyond			EoT/ EoS	Follow Up [x]
		Day -8 to Day -2	Day 1	Day 8	Day 15	Day 15-21	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15		
immunophenotyping [q]													only)				
Blood sample for RNA [r]	X		X3		X3		X			X			X (Cycles 4, 9, 15 only)				
Blood sample for receptor sequencing [s]	X						X			X			X (Cycles 4, 9, 15 only)				
Plasma sample for cell-free DNA [t]	X		X				X			X			X (Cycles 4, 9, 15 only)				
Buccal epithelial cells sample for DNA [u]	X																
Blood sample for single cell RNA sequencing [v]	X				X2												
Plasma sample for TAK-676 PK [w]			X4		X4												

AE: adverse event; CR: complete response; CT: computed tomography; CTCAE: Common Terminology Criteria for Adverse Events; CxDx: Cycle x Day x; ECG: electrocardiogram; ECOG: Eastern Cooperative Oncology Group; eCRF: electronic case report form; EOI: end of infusion; EoS: End of Study; EoT: End of Treatment; FFPE: formalin-fixed, paraffin-embedded; GI: gastrointestinal; ICF: informed consent form; IV: intravenous(ly); RECIST: Response Evaluation Criteria in Solid Tumors; MRI: magnetic resonance imaging; MUGA: multiple-gated acquisition; PD: progressive disease; PD-1: programmed cell death protein 1; PI: principal investigator; PK: pharmacokinetic(s); PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; RT: radiation therapy; SA: single agent; SAE: serious adverse event; STING: Stimulator of Interferon Genes; X2: 2 samples on the day; X3: 3 samples on the day; X4: 4 samples on the day.

Tests and procedures should be performed on schedule, but occasional changes are allowable (±3 days) for holidays, vacations, and other administrative reasons. **If extenuating circumstances prevent a patient from beginning treatment or completing a scheduled procedure or assessment within this time, the patient may continue the study only with the written permission of the medical monitor.**

[a] Unless otherwise noted, the screening visit (first screening procedure completed) must occur within 28 days before the day of the first dose of radiation. The ICF may be signed more than 28 days before the first dose of radiation. Study site must record patient data in IRT.

Table 1 SOE for Treatment With Radiation Therapy + Pembrolizumab + TAK-676 (21-Day Cycle)

	Screening [a]	RT	Cycle 1				Cycle 2			Cycle 3			Cycle 4 and Beyond			EoT/ EoS	Follow Up [x]
		Day -8 to Day -2	Day 1	Day 8	Day 15	Day 15-21	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15		

[b] Medical history will include smoking/vaping history and available baseline disease characteristics, such as disease type, staging, and PD-L1 expression. Complete physical examination (including assessment of the following systems: skin, head, eyes, ears, nose, throat, respiratory, cardiovascular, GI, neurological condition, blood and lymphatic, and musculoskeletal) will be collected at screening. The -C1D1 physical examination and medical history are not required if the screening physical examination was conducted and medical history obtained within 3 days before administration of study drug(s). Postscreening physical examinations will be symptom- or finding-directed and will be performed predose and at any time point based on clinical need. On dosing days in Cycle 1, an additional postdose physical examination will include an assessment of the respiratory and neurologic systems.

[c] Weight will be collected at screening and predose on days specified.

[d] Vital sign measurements (heart rate, blood pressure, temperature, respiratory rate, oxygen saturation) should be performed as specified and should be performed before the first fraction of radiation therapy. During Cycle 1, vital signs will be measured immediately before the start of the pembrolizumab infusion and before the start of TAK-676 infusion (within 30 minutes), 30 minutes after start of TAK-676 infusion (±5 minutes), at the EOI (±10 minutes), 2 hours postinfusion (±30 minutes), any time when clinically indicated including if the patient develops any signs or symptoms associated with potential risks of TAK-676 and/or pembrolizumab, and before discharge (if different from and not overlapping with the timepoints above).

[e] ECOG performance status should be performed before the first fraction of radiation therapy.

[f] Safety ECGs should be performed at screening and C1D1 predose. A qualified person will interpret the ECGs locally. Additional ECGs may be obtained as clinically indicated at any time during the study at the discretion of the investigator. When the timing of ECG or vital signs measurements coincides with the timing of a blood draw (eg, PK sample), the ECG measurements and vital signs measurements should be completed first, followed by blood sampling.

[g] Radiographic assessments including CT with contrast (or MRI if clinically indicated) of the disease burden will be made at screening/baseline (within 28 days before the first dose of radiation) and will be performed to assess disease response using RECIST v.1.1. criteria once at end of Cycle 2 (Day 15 to 22 [predose]), then at the end of every 3 cycles (Days 15 to 22 [predose]) up to the end of the first year, and then at the end of every 6 cycles (+7 days) thereafter until PD or the start of alternative therapies. If the patient has had an appropriate CT or MRI scan performed within 28 days of the first dose of radiation, the results of that scan may be used for tumor lesion measurements at screening.

[h] The radiation must be administered between Day -8 and Day -2 with a minimum of 40 hours between the last fraction of radiation and initiation of pembrolizumab. Note:There is no Day 0. For more information, refer to Section 8.4.2.

[i] Hematology, chemistry, thyroid function and C-reactive protein, CPK, and ferritin blood samples for C1D1 may be collected within 3 days before dosing to ensure patient eligibility on study Day 1. Repeat baseline chemistry and hematology labs should be obtained on the day of first dose of radiation if more than 2 weeks have elapsed between screening labs and the first dose of radiation.

[j] TAK-676 will be administered IV on Days 1, 8, and 15 of a 21-day cycle. On Day 1 of each cycle, TAK-676 will be administered 1 hour (+15 minutes) after the

Table 1 SOE for Treatment With Radiation Therapy + Pembrolizumab + TAK-676 (21-Day Cycle)

	Screening [a]	RT	Cycle 1				Cycle 2			Cycle 3			Cycle 4 and Beyond			EoT/ EoS	Follow Up [x]
		Day -8 to Day -2	Day 1	Day 8	Day 15	Day 15-21	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15		

end of Pembrolizumab IV infusion. The option to modify the schedule of drug administration will be based on review of the available safety and other clinical data by the investigators and the sponsor.

[k] Dosing of pembrolizumab will be a minimum 40 hours after the last fraction of radiation. Note: There is no Day 0.

[l] A serum or urine pregnancy test will be performed for women of childbearing potential at screening, on Day 1 of each cycle, and at the EOT assessment. A serum or urine pregnancy test must be performed before the first fraction of radiation therapy or predose on C1D1 if no pregnancy test had been performed within the prior 10 days with negative results available.

[m] Thyroid function tests will be performed at screening, Cycle 1, every other cycle (eg, C3, 5, 7, 9, 11) and EOT.

[n] Urinalysis samples will be collected at screening and predose on Day 1 of each cycle and analyzed at the site's local laboratory.

[o] Patients enrolling at dose levels of TAK-676 which have been shown to have pharmacodynamic activity in ongoing clinical studies will have mandatory tumor biopsies performed at screening and on Cycle 1 Days 15 through 21, between 4 and 144 hours post-TAK-676 administration. Tumor biopsies are optional, but encouraged from patients enrolling at lower dose levels. The tumor tissue biopsy at screening should be performed at least 2 days after the last dose of any prior antineoplastic therapy and within 3 weeks before the first dose of radiation. Screening biopsy should be performed only when the patient is considered otherwise eligible. The tumor biopsies should be performed on a nonirradiated site. Patients with a tumor biopsy obtained less than or equal to 3 months before initiation of TAK-676 (Day 1), will not be required to undergo a repeat pretreatment biopsy, assuming: 1) adequate tumor tissue is available for analysis (FFPE block preferred) and 2) no additional antineoplastic agents were administered since the time of the biopsy.

[p] Plasma samples to monitor changes in peripheral biomarkers, including cytokines and chemokines should be collected at screening, predose on Day 1 of indicated cycles, and during cycle 1 at the additional timepoints specified in Table 2 to monitor effects of TAK-676 on Day 1 and Day 15.

[q] Blood samples to analyze the presence and changes in immune cell state by immunophenotyping should be collected at screening and predose on Day 1 of indicated cycles. In cycle 1 only, there are an additional collections at 6 hours post-EOI of TAK-676 on C1D1 and C1D15 and predose on C1D15.

[r] Blood samples for RNA to monitor gene expression changes should be collected at screening, predose on Day 1 of indicated cycles, and during cycle 1 at the additional timepoints specified in Table 2 to monitor effects of TAK-676 on Day 1 and Day 15.

[s] Blood samples for T cell receptor and B cell receptor sequencing, to monitor induction of adaptive and humoral immune response, should be collected at screening and predose on Day 1 of indicated cycles.

[t] Blood samples for evaluation of cell-free DNA will be collected at screening and predose on Day 1 of indicated cycles. Circulating tumor DNA from these samples may be evaluated for mutations.

[u] A buccal epithelial cell sample for DNA will be obtained at screening and may be used to genotype patients for mutations or polymorphisms in genes related to

Table 1 SOE for Treatment With Radiation Therapy + Pembrolizumab + TAK-676 (21-Day Cycle)

	Screening [a]	RT	Cycle 1				Cycle 2			Cycle 3			Cycle 4 and Beyond			EoT/ EoS	Follow Up [x]
		Day -8 to Day -2	Day 1	Day 8	Day 15	Day 15-21	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15		

immune response, DNA damage repair pathways, STING gene (*TMEM173*), and drug transporters relevant to TAK-676. This sample may be collected during the course of the study if the sample cannot be collected at screening.

[v] To be collected from patients only at select centers. Screening sample should be collected only when the patient is considered otherwise eligible.

[w] Plasma samples for PK analysis of TAK-676 will be collected as specified in Table 2. The PK collection schedule may be modified based on data from ongoing studies of TAK-676, without the need for a protocol amendment.

[x] Patients who discontinue study treatment for reasons other than PD will continue follow-up every 12 ±1 weeks from the EOT visit until the occurrence of PD, loss to follow-up, consent withdrawal, the start of subsequent systemic antineoplastic therapy, study termination, or death, whichever occurs first.

Table 2 TAK-676 Biomarker Collection and PK Sample Collection

	Screening	C1D1				C1D15				C1D15-21	C2D1, C3D1, C4D1 C9D1 and C15D1
		Predose Within 30 min Before Dose [a]	EOI of TAK-676 (±10 min)	3 h Post-EOI of TAK-676 ±30 min	6 h Post-EOI of TAK-676 ±1 h	Predose Within 30 min Before Dose	EOI of TAK-676 (±10 min)	3 h Post-EOI of TAK-676 ±30 min	6 h Post-EOI of TAK-676 ±1 h		Predose Within 30 min Before Dose
Archival (banked) tumor tissue sample	X										
Fresh tumor tissue biopsy sample [b]	X									X	
Plasma sample for circulating biomarkers	X	X		X	X	X		X	X		X
Blood sample for immunophenotyping	X	X			X	X			X		X
Blood sample for RNA	X	X		X	X	X		X	X		X
Blood sample for receptor sequencing	X										X
Plasma sample for cell-free DNA	X	X									X
Buccal epithelial cells sample for DNA	X										
Blood sample for single cell RNA seq [c]	X					X			X		
Plasma sample for TAK-676 PK [d]		X	X	X	X	X	X	X	X		

CxDx: Cycle x, Day x; EOI: end of infusion; FFPE: fixed-formalin, paraffin-embedded; PK: pharmacokinetic(s).

Table 2 TAK-676 Biomarker Collection and PK Sample Collection

	Screening	C1D1				C1D15				C1D15-21	C2D1, C3D1, C4D1 C9D1 and C15D1
		Predose Within 30 min Before Dose [a]	EOI of TAK-676 (±10 min)	3 h Post-EOI of TAK-676 ±30 min	6 h Post-EOI of TAK-676 ±1 h	Predose Within 30 min Before Dose	EOI of TAK-676 (±10 min)	3 h Post-EOI of TAK-676 ±30 min	6 h Post-EOI of TAK-676 ±1 h		Predose Within 30 min Before Dose

[a] The predose sample collections will be conducted before pembrolizumab and TAK-676 administration. All other timeframes within the table should be relative to TAK-676 administration, not pembrolizumab. The timing of the morning visits should occur at approximately the same time as previous dosing days. The date/time of the start and end of infusions should be recorded accurately.

[b] Patients enrolling at dose levels of TAK-676 which have been shown to have pharmacodynamic activity in ongoing clinical studies will have mandatory tumor biopsies performed at screening and on Cycle 1 Days 15 through 21, between 4 and 144 hours post-TAK-676 administration. Tumor biopsies are optional but encouraged from patients enrolling at lower dose levels. The tumor tissue biopsy at screening should be performed at least 2 days after the last dose of any prior antineoplastic therapy and within 3 weeks before the first dose of radiation. Screening biopsy should be performed only when the patient is considered otherwise eligible. The tumor biopsies should be performed on a nonirradiated site. Patients with a tumor biopsy obtained less than or equal to 3 months before initiation of TAK-676 (Day 1), will not be required to undergo a repeat pretreatment biopsy, assuming: 1) adequate tumor tissue is available for analysis (FFPE block preferred) and 2) no additional antineoplastic agents were administered since the time of the biopsy.

[c] To be collected from patients only at select centers at screening and on C1D15 predose and C1D15 6 hours post dose. Screening sample should be collected only when the patient is considered otherwise eligible.

[d] PK samples are always collected from the contralateral arm (not the same arm as infusion arm).

Appendix B Responsibilities of the Investigator

Clinical research studies sponsored by the sponsor are subject to ICH GCP and all the applicable local laws and regulations. The responsibilities imposed on investigators by the FDA are summarized in the Statement of Investigator (Form FDA 1572), which must be completed and signed before the investigator may participate in this study.

The investigator agrees to assume the following responsibilities by signing a Form FDA 1572:

1. Conduct the study in accordance with the protocol.
2. Personally conduct or supervise the staff who will assist in the protocol.
3. If the investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the investigator/institution should ensure that this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.
4. Ensure that study-related procedures, including study-specific (nonroutine/nonstandard panel) screening assessments, are NOT performed on potential subjects before the receipt of written approval from relevant governing bodies/authorities.
5. Ensure that all colleagues and employees assisting in the conduct of the study are informed of these obligations.
6. Secure prior approval of the study and any changes by an appropriate IRB/IEC that conform to 21 CFR Part 56, ICH and local regulatory requirements.
7. Ensure that the IRB/IEC will be responsible for initial review, continuing review, and approval of the protocol. Promptly report to the IRB/IEC all changes in research activity and all anticipated risks to subjects. Make at least yearly reports on the progress of the study to the IRB/IEC, and issue a final report within 3 months of study completion.
8. Ensure that requirements for informed consent, as outlined in 21 CFR Part 50, ICH and local regulations, are met.
9. Obtain valid informed consent from each subject who participates in the study, and document the date of consent in the subject's medical chart. Valid informed consent is the most current version approved by the IRB/IEC. Each ICF should contain a subject authorization section that describes the uses and disclosures of a subject's personal information (including personal health information) that will take place in connection with the study. If an ICF does not include such a subject authorization, then the investigator must obtain a separate subject authorization form from each subject or the subject's legally acceptable representative.
10. Prepare and maintain adequate case histories of all persons entered into the study, including eCRFs, hospital records, laboratory results, etc., and maintain these data for a minimum of 2 years following notification by the sponsor that all investigations have been discontinued or that the regulatory authority has approved the marketing application. The investigator should

contact and receive written approval from the sponsor before disposing of any such documents.

11. Allow possible inspection and copying by the regulatory authority of GCP-specified essential documents.
12. Maintain current records of the receipt, administration, and disposition of sponsor-supplied drugs, and return all unused sponsor-supplied drugs to the sponsor.
13. Report adverse reactions to the sponsor promptly. In the event of an SAE, notify the sponsor within 24 hours.

Appendix C Investigator Consent to Use of Personal Information

Takeda will collect and retain personal information of the investigator, including his or her name, address, and other identifying personal information. In addition, the investigator's personal information may be transferred to other parties located in countries throughout the world (eg, the UK, US, and Japan), including the following:

- Takeda, its affiliates, and licensing partners.
- Business partners assisting Takeda, its affiliates, and licensing partners.
- Regulatory agencies and other health authorities.
- IRBs and IECs.

The investigator's personal information may be retained, processed, and transferred by Takeda and these other parties for research purposes including the following:

- Assessment of the suitability of the investigator for the study and/or other clinical studies.
- Management, monitoring, inspection, and audit of the study.
- Analysis, review, and verification of the study results.
- Safety reporting and pharmacovigilance relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to other medications used in other clinical studies that may contain the same chemical compound present in the study medication.
- Inspections and investigations by regulatory authorities relating to the study.
- Self-inspection and internal audit within Takeda, its affiliates, and licensing partners.
- Archiving and audit of study records.
- Posting investigator site contact information, study details, and results on publicly accessible clinical trial registries, databases, and websites.

The investigator's personal information may be transferred to other countries that do not have data protection laws that offer the same level of protection as data protection laws in the investigator's own country.

The investigator acknowledges and consents to the use of his or her personal information by Takeda and other parties for the purposes described above.

Appendix D ECOG Scale for Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all predisease performance without restriction.
1	Symptoms but ambulatory. Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Source: (Oken et al. 1982).
ECOG: Eastern Cooperative Oncology Group.

Appendix E BOIN Design

The Bayesian Optimal INterval (BOIN) design (Liu and Yuan 2015) will be used to guide the dose escalation decisions and MTD estimation. This trial was designed and will be conducted using the software BOIN that is available at www.trialdesign.org.

The target toxicity rate is 0.3. During the study, if the observed DLT rate at the current dose is ≤0.236, the next cohort of patients will be treated at the next higher dose level; if it is ≥0.358, the next cohort will be treated at the next lower dose level; if it is within 0.236 and 0.358, additional patients will be enrolled in this dose level. For the purpose of overdose control, a dose level and all the dose levels above it will be eliminated from further examination if its posterior probability of exceeding the target toxicity rate is greater than 0.95. If the lowest dose level is eliminated, stop the escalation for safety. Unless such early stop happens, dose escalation will continue until the maximum sample size is reached, or escalation will be stopped if the number of patients treated at the current dose reaches 9 and the recommendation is to retain at the current dose level. The dose escalation algorithm is described as the following steps:

Step 1. Patients in the first cohort are treated at lowest dose level.

Step 2. Decide the dose level for the next cohort according to the rule shown in Table 1. Please note the following when using Table 1:

1. "Eliminate" means that the current dose level and all higher dose levels are eliminated to prevent treating any future patients at these overly toxic dose levels.
2. When a dose level is eliminated, automatically de-escalate to the next lower level. When the lowest dose level is eliminated, stop the dose escalation for safety. In this case, no dose level should be selected as the MTD.
3. If none of the actions (ie, escalation, de-escalation, or elimination) is triggered, the next cohort will be treated with the current dose.
4. If the current dose is the lowest dose level and the rule indicates a de-escalation, treat the next cohort at the lowest dose level unless the number of DLTs reaches the elimination boundary, at which point the dose escalation should be terminated for safety.
5. If the current dose is the highest dose and the rule indicates an escalation, treat the next cohort at the highest dose.

Step 3. Repeat Step 2 until the maximum sample size is reached or until a certain number of patients (n = 9) have already been treated with the current dose level and the recommendation is to retain at the current dose level.

Table 1 Dose Escalation/De-escalation Rule for the BOIN Design

Number of Patients Treated at the Current Dose	1	2	3	4	5	6	7	8	9
Escalate if number of DLT ≤	0	0	0	0	1	1	1	1	2
Deescalate if number of DLT ≥	1	1	2	2	2	3	3	3	4
Eliminate if number of DLT ≥	NA	NA	3	3	4	4	5	5	5

DLT: dose-limiting toxicity; NA: not applicable.
Number of DLTs is the number of patients with at least 1 DLT.

After the dose escalation is complete, the MTD is selected based on isotonic regression as specified in (Liu and Yuan 2015). Specifically, the dose is selected as the MTD for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, the higher dose level is selected when the isotonic estimate is lower than the target toxicity rate; the lower dose level is selected when the isotonic estimate is greater than or equal to the target toxicity rate.

The operating characteristics of the BOIN design are evaluated with simulations assuming various distributions of toxicity across dose levels shown in Table 2.

Table 2 Simulation Scenarios

	True DLT rate					
Dose Level	**Scenario 1**	**Scenario 2**	**Scenario 3**	**Scenario 4**	**Scenario 5**	**Scenario 6**
0.2 mg	**0.3**	0.12	0.06	0.05	0.04	0.02
0.4 mg	0.44	**0.3**	0.15	0.1	0.07	0.05
0.8 mg	0.5	0.44	**0.3**	0.12	0.1	0.08
1.2 mg	0.55	0.5	0.45	**0.3**	0.12	0.11
1.6 mg	0.6	0.55	0.51	0.45	**0.3**	0.14
2.0 mg	0.65	0.6	0.57	0.51	0.47	**0.3**
2.5 mg	0.7	0.65	0.64	0.62	0.59	0.48

DLT: dose-limiting toxicity.

The combination of the radiation therapy schedule and IV pembrolizumab at 200 mg every 3 weeks plus TAK-676 at a range of dose levels will be evaluated in a maximum of 39 DLT-evaluable patients with locally advanced or metastatic NSCLC, TNBC, or SCCHN. For simulation purpose, we will consider dose levels from 0.2 mg to 2.5 mg (when additional dose levels are explored, they may be evaluated for operating characteristics in the Statistical Analysis Plan). Each cohort will have 3 patients. The operating characteristics calculated from the simulations are shown in Table 3.

Table 3 Operating Characteristics

	Scenario 1		Scenario 2		Scenario 3	
Dose Level (TAK-676)	**Selection %**	**% Pts Treated**	**Selection %**	**% Pts Treated**	**Selection %**	**% Pts Treated**
0.2 mg	**61.8**	**61.8**	20.5	31.8	2.4	16.8
0.4 mg	20.9	20.9	**56.4**	**41.4**	26.0	28.5
0.8 mg	3.8	3.8	18.3	20.5	**50.8**	**33.7**
1.2 mg	0.4	0.4	4.0	5.4	16.6	16.3
1.6 mg	0.0	0.0	0.4	0.8	3.6	3.9
2.0 mg	0.0	0.0	0.0	0.1	0.6	0.8
2.5 mg	0.0	0.0	0.0	0.0	0.0	0.1
% Early Stopping	13.1		0.4		0.0	
Expected # of Pts	14.9		21.4		23.9	
	Scenario 3		**Scenario 4**		**Scenario 6**	
Dose Level (TAK-676)	**Selection %**	**% Pts Treated**	**Selection %**	**% Pts Treated**	**Selection %**	**% Pts Treated**
0.2 mg	0.7	13.2	0.3	11.4	0	10.2
0.4 mg	4.4	15.7	1.4	12.7	0.7	11.2
0.8 mg	18	22.9	4.4	14	2.1	12.4
1.2 mg	**57.9**	**30.6**	20.7	20.9	4.4	13.8
1.6 mg	15.7	14.4	**55.2**	**26.5**	23.2	19.1
2.0 mg	3	2.7	16.6	12.6	**52.5**	**22.7**
2.5 mg	0.2	0.5	1.4	2	17.1	10.5
% Early Stopping	0.0		0.0		0.0	
Expected # of Pts	27.5		29.9		31.9	

Appendix F Cockcroft-Gault Equation

For male patients:

$$\text{Creatinine clearance} = \frac{(140 - \text{age[years]}) \times \text{weight [kg]}}{72 \times (\text{serum creatinine[mg/dL]})}$$

For female patients:

$$\text{Creatinine clearance} = \frac{0.85\ (140 - \text{age[years]}) \times \text{weight [kg]}}{72 \times (\text{serum creatinine[mg/dL]})}$$

Source: (Cockcroft and Gault 1976).

Appendix G New York Heart Association Classification of Cardiac Disease

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. Ninth Ed. Boston, MA: Little, Brown & Co; 1994:253-256 (The Criteria Committee of New York Heart Association 1994).

Appendix H Response Evaluation Criteria in Solid Tumors (RECIST Version 1.1)

All sites of disease, target, and nontarget lesions must be assessed at baseline. Objective disease status is to be recorded at each evaluation using the response categories and definitions provided in this section.

All sites of measurable lesions up to a maximum of 2 lesions per organ, 5 lesions in total, representative of all involved organs, should be identified as target lesions at baseline. Target lesions should be selected based on size (longest lesions) and suitability for reproducible repeated measurements. Measurements must be provided for target site of measurable lesions.

Disease Response Criteria for Target and Nontarget Lesions

Evaluation of Target Lesions	
CR:	Disappearance of all target lesions. Any pathological lymph nodes (whether target or nontarget) must have reduction in short axis to <10 mm.
PR:	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.
PD:	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of 1 or more new lesions. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.
SD:	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.
Evaluation of Nontarget Lesions	
CR:	Disappearance of all nontarget lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).
Non-CR/Non-PD:	Persistence of 1 or more nontarget lesion(s) or/and maintenance of tumor marker level above the normal limits.
PD:	Unequivocal progression of existing non-target lesions. (Note: the appearance of 1 or more new lesions is also considered progression).

Source: (Eisenhauer et al. 2009).

CR: complete response; LD: longest diameter; PD: progressive disease; PR: partial response; SD: stable disease.

The following table summarizes the overall response status calculation at each time point for patients who have measurable disease per RECIST v.1.1 at baseline.

Time Point Response: Patients With Target (± Nontarget) Disease

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Source: (Eisenhauer et al. 2009).

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; NE: not evaluable.

The following table summarizes the overall response status calculation at each time point for patients who have nonmeasurable (therefore nontarget) disease at baseline.

Time Point Response: Patients with Nontarget Disease Only

Nontarget Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD [a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Source: (Eisenhauer et al. 2009).

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; NE: not evaluable.

[a] Non-CR/non-PD is preferred over stable disease for nontarget disease because SD is increasingly used as endpoint for assessment of efficacy in some study so to assign this category when no lesions can be measured is not advised.

Special Allowances to RECIST v.1.1: As described in Section 9.4.14.1, accumulating evidence indicates a minority of patients treated with immunotherapy may derive clinical benefit despite initial evidence of PD. Patients will be permitted to continue study treatment beyond initial RECIST v.1.1 defined PD as long as they meet the criteria outlined in Section 9.4.14.1. All decisions to continue treatment beyond initial progression must be discussed with the medical monitor and documented in the study records.

Appendix I Clinical Inhibitors of OATP1B1 and OATP1B3

Drug Class	Medication Name (Brand Name) [a]	Required Washout Period Before First Dose
Antiviral	atazanavir (Reyataz) lopinavir (Kaletra) ritonavir (Norvir) simeprevir (Olysio) remdesivir (Veklury)	14 days before first dose of study drug(s)
Antibiotic	clarithromycin (Biaxin, Biaxin XL) erythromycin (E.E.S. Granules, E.E.S.-400 Filmtab, EryPed 200, EryPed 400, Ery-Tab, Erythrocin Lactobionate, Erythrocin Stearate Filmtab, PCE Dispertab) rifampin (single dose)/rifampicin (Rifadin, Rifadin IV, Rimactane)	
Immunosuppressant	cyclosporine (Gengraf, Neoral, SandIMMUNE)	
Antilipemic/ hypercholesterolemia	gemfibrozil (Lopid)	

Sources: fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf and fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.
DDI: drug-drug interaction; FDA: [United States] Food and Drug Administration; OATP: organic anion-transporting polypeptide.
[a] Note the list of clinical inhibitors of OATP1B1 and OATP1B3 is not exhaustive and is based on the FDA Draft DDI Guidance.

Appendix J Dose constraints as per Task Group-101

		One fraction		Three fractions		Five fractions		
Serial tissue	**Max critical volume above threshold**	**Threshold dose (Gy)**	**Max point dose (Gy)[a]**	**Threshold dose (Gy)**	**Max point dose (Gy)[a]**	**Threshold dose (Gy)**	**Max point dose (Gy)[a]**	**End point (>Grade 3)**
Optic pathway	<0.2 cc	8	10	15.3 (5.1 Gy/fx)	17.4 (5.8 Gy/fx)	23 (4.6 Gy/fx)	25 (5 Gy/fx)	Neuritis
Cochlea			9		17.1 (5.7 Gy/fx)		25 (5 Gy/fx)	Hearing loss
Brainstem (not medulla)	<0.5 cc	10	15	18 (6 Gy/fx)	23.1 (7.7 Gy/fx)	23 (4.6 Gy/fx)	31 (6.2 Gy/fx)	Cranial neuropathy
Spinal cord and medulla	<0.35 cc	10	14	18 (6 Gy/fx)	21.9 (7.3 Gy/fx)	23 (4.6 Gy/fx)	30 (6 Gy/fx)	Myelitis
	<1.2 cc	7		12.3 (4.1 Gy/fx)		14.5 (2.9 Gy/fx)		
Spinal cord subvolume (5–6 mm above and below level treated per Ryu)	<10% of subvolume	10	14	18 (6 Gy/fx)	21.9 (7.3 Gy/fx)	23 (4.6 Gy/fx)	30 (6 Gy/fx)	Myelitis
Cauda equina	<5 cc	14	16	21.9 (7.3 Gy/fx)	24 (8 Gy/fx)	30 (6 Gy/fx)	32 (6.4 Gy/fx)	Neuritis
Sacral plexus	<5 cc	14.4	16	22.5 (7.5 Gy/fx)	24 (8 Gy/fx)	30 (6 Gy/fx)	32 (6.4 Gy/fx)	Neuropathy
Esophagus [b]	<5 cc	11.9	15.4	17.7 (5.9 Gy/fx)	25.2 (8.4 Gy/fx)	19.5 (3.9 Gy/fx)	35 (7 Gy/fx)	Stenosis/fistula
Brachial plexus	<3 cc	14	17.5	20.4 (6.8 Gy/fx)	24 (8 Gy/fx)	27 (5.4 Gy/fx)	30.5 (6.1 Gy/fx)	Neuropathy
Heart/pericardium	<15 cc	16	22	24 (8 Gy/fx)	30 (10 Gy/fx)	32 (6.4 Gy/fx)	38 (7.6 Gy/fx)	Pericarditis
Great vessels	<10 cc	31	37	39 (13 Gy/fx)	45 (15 Gy/fx)	47 (9.4 Gy/fx)	53 (10.6 Gy/fx)	Aneurysm
Trachea and large bronchus [b]	<4 cc	10.5	20.2	15 (5 Gy/fx)	30 (10 Gy/fx)	16.5 (3.3 Gy/fx)	40 (8 Gy/fx)	Stenosis/fistula

		One fraction		Three fractions		Five fractions		
		Threshold dose (Gy)	**Max point dose (Gy)[a]**	**Threshold dose (Gy)**	**Max point dose (Gy)[a]**	**Threshold dose (Gy)**	**Max point dose (Gy)[a]**	**End point (>Grade 3)**
Bronchus-smaller airways	<0.5 cc	12.4	13.3	18.9 (6.3 Gy/fx)	23.1 (7.7 Gy/fx)	21 (4.2 Gy/fx)	33 (6.6 Gy/fx)	Stenosis with atelectasis
Rib	<1 cc	22	30	28.8 (9.6 Gy/fx)	36.9 (12.3 Gy/fx)	35 (7 Gy/fx)	43 (8.6 Gy/fx)	Pain or fracture
	<30 cc			30.0 (10.0 Gy/fx)				
Skin	<10 cc	23	26	30 (10 Gy/fx)	33 (11 Gy/fx)	36.5 (7.3 Gy/fx)	39.5 (7.9 Gy/fx)	Ulceration
Stomach	<10 cc	11.2	12.4	16.5 (5.5 Gy/fx)	22.2 (7.4 Gy/fx)	18 (3.6 Gy/fx)	32 (6.4 Gy/fx)	Ulceration/fistula
Duodenum [b]	<5 cc	11.2	12.4	16.5 (5.5 Gy/fx)	22.2 (7.4 Gy/fx)	18 (3.6 Gy/fx)	32 (6.4 Gy/fx)	Ulceration
	<10 cc	9		11.4 (3.8 Gy/fx)		12.5 (2.5 Gy/fx)		
Jejunum/ileum [b]	<5 cc	11.9	15.4	17.7 (5.9 Gy/fx)	25.2 (8.4 Gy/fx)	19.5 (3.9 Gy/fx)	35 (7 Gy/fx)	Enteritis/ obstruction
Colon [b]	<20 cc	14.3	18.4	24 (8 Gy/fx)	28.2 (9.4 Gy/fx)	25 (5 Gy/fx)	38 (7.6 Gy/fx)	Colitis/fistula
Rectum [b]	<20 cc	14.3	18.4	24 (8 Gy/fx)	28.2 (9.4 Gy/fx)	25 (5 Gy/fx)	38 (7.6 Gy/fx)	Proctitis/fistula
Bladder wall	<15 cc	11.4	18.4	16.8 (5.6 Gy/fx)	28.2 (9.4 Gy/fx)	18.3 (3.65 Gy/fx)	38 (7.6 Gy/fx)	Cystitis/fistula
Penile bulb	<3 cc	14	34	21.9 (7.3 Gy/fx)	42 (14 Gy/fx)	30 (6 Gy/fx)	50 (10 Gy/fx)	Impotence
Femoral heads (right and left)	<10 cc	14		21.9 (7.3 Gy/fx)		30 (6 Gy/fx)		Necrosis
Renal hilum/vascular trunk	<2 - 3 volume	10.6	18.6 (6.2 Gy/fx)			23 (4.6 Gy/fx)		Malignant hypertension
Parallel tissue	**Minimum critical volume below threshold**							
Lung (right and left)	1500 cc	7	NA-Parallel tissue	11.6 (2.9 Gy/fx)	NA-Parallel tissue	12.5 (2.5 Gy/fx)	NA-Parallel tissue	Basic lung function

		One fraction		Three fractions		Five fractions		
		Threshold dose (Gy)	**Max point dose (Gy)[a]**	**Threshold dose (Gy)**	**Max point dose (Gy)[a]**	**Threshold dose (Gy)**	**Max point dose (Gy)[a]**	**End point (>Grade 3)**
Lung (right and left)	1000 cc	7.4	NA-Parallel tissue	12.4 (3.1 Gy/fx)	NA-Parallel tissue	13.5 (2.7 Gy/fx)	NA-Parallel tissue	Pneumonitis
Liver	700 cc	9.1	NA-Parallel tissue	19.2 (4.8 Gy/fx)	NA-Parallel tissue	21 (4.2 Gy/fx)	NA-Parallel tissue	Basic liver function
Renal cortex (right and left)	200 cc	8.4	NA-Parallel tissue	16 (4 Gy/fx)	NA-Parallel tissue	17.5 (3.5 Gy/fx)	NA-Parallel tissue	Basic renal function

Source: (Benedict et al. 2010).

[a] "Point" defined as 0.035 cc or less.

[b] Avoid circumferential irradiation.

Appendix K Statistical Considerations for Paired Biopsies

While biopsies are requested of all patients with safely accessible nonirradiated/abscopal lesions (in the opinion of the investigator), safely accessible biopsies will become mandatory once evidence of peripheral immune activation is observed during dose escalation. Pending successful completion of the dose escalation phase of the study, it is anticipated that additional patients will be evaluated in future dose expansion. In the future dose expansion cohort, we hope to demonstrate a biologically and statistically meaningful increase of T-cell infiltration into tumors after treatment of TAK-676 + pembrolizumab + radiation. A certain number of paired patient biopsies (from a single indication and dose level) in the escalation and expansion cohorts may be needed to achieve our desired statistical confidence. To evaluate that, we surveyed the literature for the level of T-cell infiltration into tumors treated with immune oncology agents in various tumor types (Chalabi et al. 2020; Ferrarotto et al. 2020; Steele et al. 2018; Tumeh et al. 2014). Evaluation of published results suggest that CPI treatment-mediated T-cell infiltration can result in approximately 1.5- to 3-fold increases in CD8 T cells within the tumor microenviroment via the evaluation of pretreatment and posttreatment biopsies and yields an estimated coefficient of variation of 80% to 180% in majority of the studies. On the basis of these data, we assumed a 2.5-fold increase and a coefficient of variation of 130% to project the minimum number of paired biopsies that are needed to achieve a 0.8 posterior probability of achieving a 1.5-fold increase in T cells after treatment, with a probability of success of 80%. On the basis of these assumptions, we projected that 11 pairs of high quality and interpretable biopsies are needed. The total number of patients biopsied within an indication needed to support this statistical goal is expected to be greater than 11 patients and will be based on biopsy quality and interpretability (refer to Statistical Analysis Plan for additional details).

Appendix L Protocol History

Date	Amendment Number	Region
18 February 2022	Amendment 2	North America
31 March 2021	Amendment 1	North America
23 October 2020	Initial protocol	North America

Rationale for Amendment 1

This document describes the changes to the protocol incorporating Amendment 1.

The primary reason for this amendment is to clarify concepts and criteria for treatment as well as response assessments.

Minor grammatical, editorial, formatting, and administrative changes not affecting the conduct of the study are included for clarification and administrative purposes only.

Protocol Amendment 1			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
1	Section 2.0 STUDY SUMMARY Secondary Objectives Section 5.1.2 Secondary Objectives	In the objective describing antitumor activity, replaced the term "abscopal effect" with "nonirradiated lesions."	Updated text throughout protocol from "abscopal" to "nonirradiated" and from "local" to "irradiated" for a more accurate description, since patients are also being treated with systemic therapies as opposed to just radiation therapy.
2	Section 2.0 STUDY SUMMARY Inclusion Criteria Section 4.5.1 Rationale for the Selected Patient Population Section 7.1 Inclusion Criteria	Updated Inclusion Criterion 5 to clarify that patients must have received or been offered all established standard of care (SOC) treatment options for which they were eligible in addition to having progressed on checkpoint inhibitors in a prior line of therapy.	Clarification to address Central Institutional Review Board inquiry.

Protocol Amendment 1			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
3	Section 2.0 STUDY SUMMARY Exclusion Criteria Section 7.2 Exclusion Criteria	Updated Exclusion Criterion 13 to provide examples of live attenuated virus.	Clarification with examples.
4	Section 2.0 STUDY SUMMARY Main Criteria for Evaluation and Analyses Statistical Considerations Section 3.3 List of Abbreviations Section 5.2.2 Secondary Endpoints Table 6.b Primary and Secondary Endpoints for Disclosures	Renamed response assessments to replace "local" or "localized" with "irradiated" and to replace "abscopal" with "nonirradiated" as follows: irradiated response rate (ORRirradiated); nonirradiated response rate (ORRnonirradiated); confirmed complete response (cCRirradiated) and confirmed partial response (cPRirradiated) of tumor lesions lying within the radiation field; confirmed complete response (cCRnonirradiated) and confirmed partial response (cPRnonirradiated) of tumor lesions lying outside of the radiation field; duration of response for tumors lying within the radiation field (DORirradiated) and for those lying outside of the radiation field (DORnonirradiated); and time to response for tumors lying within the radiation field (TTRirradiated), and for those lying outside of the radiation field (TTRnonirradiated).	Since patients are being treated with systemic therapies, changed "abscopal" to "nonirradiated" and "local" to "irradiated" for a more accurate description.
5	Section 2.0 STUDY SUMMARY Statistical Considerations Section 6.1 Overview of Study Design Section 6.1.1 Dose Escalation of TAK-676 Section 8.3 Dose Escalation Rules	Added the following clarifying text: It is generally expected that at least 3 patients will enroll per cohort. However, if no DLTs have been identified in the TAK-676 + pembrolizumab dose level that has already been evaluated in the dose-finding phase 1 TAK-676-1002 study, the sponsor, in agreement with the TAK-676-1003 investigators, may opt to enroll a 2-patient cohort at that same [radiation] + TAK-676 + pembrolizumab dose level being evaluated in the TAK-676-1003 study.	Clarification of dose escalation procedures.

Protocol Amendment 1			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
6	Section 5.2.3 Exploratory Endpoints Section 13.1.5 Pharmacodynamic Analysis	Added changes in protein expression to the exploratory endpoint of changes between screening and on-treatment tumor biopsies in immune contexture and gene expression.	Clarification.
7	Section 6.1.1 Dose Escalation of TAK-676	Clarified that replacement of dose-limiting toxicity (DLT)-inevaluable patients during dose escalation may not be mandatory except for the first cohort. Replacement may be determined based on the number of DLT-evaluable patients in the cohort.	Clarification.
8	Section 6.2 Number of Patients	Deleted the following sentence: "During the dose escalation phases, patients not receiving all required doses in Cycle 1 for reasons other than DLT will be replaced."	Update for consistency with patient replacement procedures.
9	Section 6.3.5 Post-trial Access	Clarified post-trial reimbursement procedures for pembrolizumab.	Clarification.
10	Section 8.1.2 Pembrolizumab	Updated that pembrolizumab dosing was to be on an every 3-week basis (rather than on a 3-week basis) after the last fraction of radiation.	Editorial update.
11	Section 8.1.3 TAK-676	Clarified the allowed interval between dosing pembrolizumab and TAK-676 and the allowable windows for TAK-676 dosing in all cycles except Cycle 1. Clarified that vital signs were to be measured before discharge if different from and not overlapping with specified timepoints for vital signs measurement.	Clarification of timing of TAK-676 administration and timing of vital signs measurement before discharge.
12	Section 8.2 Definitions of DLT Section 8.5 Excluded Concomitant Medications and Procedures	Added that coronavirus disease 2019 (COVID-19) vaccinations should be avoided during the Cycle 1 DLT window.	Guidance on allowed timing of COVID-19 vaccination.

Protocol Amendment 1			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
13	Section 8.3 Dose Escalation Rules Appendix E BOIN Design	Modified text to indicate that dose escalation will continue until the maximum sample size is reached or the number of patients treated at the current dose level reaches 9 and the recommendation is to retain at the current dose level.	Clarification to describe the BOIN escalation rule more accurately.
14	Section 8.4 Dose Modification Guidelines	Clarified that discussions and agreements regarding dose modifications will be documented and that possible dose modifications due to AEs not related to study drug should be discussed with the medical monitor.	Clarification.
15	Section 8.4.1 Criteria for Administering a Subsequent Dose/Starting a New Cycle	Clarified criteria for holding or delaying treatment in the event of failing to meet retreatment criteria. Clarified that dose modifications due to clinically significant lab values not related to study drug are not required but should be discussed and documented with the medical monitor before the next dose.	Clarification.
16	Table 8.g Guidelines for TAK-676 Dose Modification and/or Discontinuation for Hematologic Toxicity	Updated that TAK-676 should be held until absolute neutrophil count resolved to $\geq 1000/mm^3$ (instead of $\geq 1500/mm^3$) in the event of febrile neutropenia.	Correction.
17	Section 8.5 Excluded Concomitant Medications and Procedures	Added that COVID-19 vaccinations were prohibited within ±3 days of systemic study treatments and should be avoided during the Cycle 1 DLT window. Added that certain prohibited medications may be used to treat adverse events during a TAK-676 dose interruption in which there was a sufficient washout of TAK-676; these situations were to be handled on a case by case basis with discussion between the investigator and medical monitor.	Guidance on allowed timing of COVID-19 vaccination and on use of prohibited medications to treat AEs during a study drug dose interruption.

Protocol Amendment 1			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
18	Section 8.8.12 Management of COVID-19–Positive Patients	Added a section describing procedures for managing COVID-19-positive patients.	Guidance on management of patients with positive COVID-19 results.
19	Section 8.10.1 TAK-676 Section 8.10.2 Pembrolizumab	Clarified locations other than the Pharmacy Manual for information on preparation, storage, handling, and administration of the drug products.	Clarification.
20	Section 9.4 Study Procedures	Revised text to indicate that screening procedures must be completed within 28 days before the first dose of radiation, instead of within 28 days before administration of the first dose of study drug.	Clarification.
21	Section 9.4.11 Enrollment	Deleted reference to "Parts A and B" of the study.	Correction.
22	Section 9.4.14 Disease Assessment	Revised to indicated that screening radiographic disease assessments are to be completed within 28 days before the first dose of radiation.	Clarification.
23	Section 9.4.14.1 Special Allowances to RECIST v.1.1 and Treatment Beyond Progression Section 9.4.14.1.1 Irradiated and Nonirradiated Response Section 9.4.14.1.2 Treatment Beyond Progression	Added Section 9.4.14.1.1 to describe irradiated and nonirradiated response assessment and a heading for Section 9.4.14.1.2 on treatment beyond progression.	Clarified how irradiated and nonirradiated response should be assessed within the context of RECIST 1.1.
24	Table 9.c Primary Specimen Collection	Added protein as a primary specimen derivative 2 to be collected from archival tumor tissue.	Alignment with updated exploratory endpoint.
25	Section 9.7 Discontinuation of Treatment With Study Drug and Patient Replacement	Added text clarifying circumstances for discontinuation of study treatment or study participation, and discontinuation of 1 study drug while the other is continued. Clarified that replacement of DLT-inevaluable patients during dose escalation may not be mandatory and may be determined based on the number of DLT-evaluable patients in the cohort.	Since using the Bayesian Optimal Interval (BOIN) design, which does not mandate exact number of patients required in a given cohort, added flexibility re: patient replacement within a cohort.

Protocol Amendment 1			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
26	Section 9.9 Completion of Study (for Individual Patients)	Clarified that patients will be considered to have completed the study if they are discontinued from both study drugs and one of the criteria specified in the section are met. One criterion was updated as completion of follow-up period and assessments, if applicable. A statement that the end of study electronic case report form (eCRF) should be completed was added. Text from a separate section (Section 9.10 Withdrawal of Patients From the Study) was added to this section and the separate section was deleted.	Combination of similar sections to reduce redundancy and for clarification of procedures.
27	Section 10.3 Monitoring of AEs and Period of Observation	Clarified that all adverse events (AEs) were to be reported from the signing of the informed consent through 30 days after the administration of the last dose of study drug(s) or before initiation of new anticancer therapy (whichever comes first) and recorded in the eCRFs.	Clarification.
28	Section 10.6 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities	Added missing heading.	Added missing heading.
29	Section 13.1.1 Analysis Sets	Updated the definition of the DLT-evaluable analysis set to clarify that patients who receive only 1 or 2 doses of radiation will not be considered DLT-evaluable and may or may not be replaced within the same cohort and allowed to continue on study. Updated the definitions of the response-evaluable and pharmacodynamic analysis sets to add that patients must have received at least 1 dose of TAK-676.	Since using the BOIN design, which doesn't mandate exact number of patients required in a given cohort, added flexibility re: patient replacement within a cohort. Clarified that patients need to have received at least 1 dose of TAK-676 to be included in the response evaluable and pharmacodynamic analysis sets.

Protocol Amendment 1			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
30	Section 13.1.3 Efficacy Analysis	Updated the efficacy endpoint names to ORRnonirradiated, ORRirradiated, DORnonirradiated, DORirradiated, TTRnonirradiated, and TTRirradiated and updated the definition of DORnonirradiated to use "nonirradiated" instead of "abscopal."	Since patients are being treated with systemic therapies, changed "abscopal" to "nonirradiated" and "local" to "irradiated" for a more accurate description.
31	Section 13.3 Determination of Sample Size	Deleted the extra word "for" from the following: "The details of dose escalation rules for are specified in Section 8.3."	Correction.
32	Section 16.0 REFERENCES	Added new reference for Response Criteria for Intratumoral Therapy in Solid Tumors.	Update to align with added text on irradiated and nonirradiated response.
33	Appendix A Schedule of Events Table 1 SOE for Treatment With Radiation Therapy + Pembrolizumab + TAK-676 (21-Day Cycle)	Updated footnote d to add that vital signs were to be measured before discharge if different from and not overlapping with specified timepoints for vital signs measurement. Added a cross reference to footnote h (formerly footnote k) to radiation therapy administration row. Added the following text to footnote h: "Repeat baseline chemistry and hematology labs should be obtained on the day of first dose of radiation if more than 2 weeks have elapsed between screening labs and the first dose of radiation." Reversed the order of footnotes u and v as a correction.	Clarification and correction.
34	Appendix I Clinical Inhibitors of OATP1B1 and OATP1B3	Added the brand name for remdesivir.	Update for completeness.

Amendment 02 to An Open-Label, Phase 1, Dose-Escalation Study to Evaluate the Safety and Preliminary Antitumor Activity of TAK-676 With Pembrolizumab Following Radiation Therapy in the Treatment of Non-Small-Cell Lung Cancer, Triple-Negative Breast Cancer, or Squamous-Cell Carcinoma of the Head and Neck that Has Progressed on Checkpoint Inhibitors

ELECTRONIC SIGNATURES

Signed by	Meaning of Signature	Server Date (dd-MMM-yyyy HH:mm 'UTC')
[redacted]	Biostatistics Approval	21-Feb-2022 14:23 UTC
[redacted]	Clinical Pharmacology Approval	21-Feb-2022 14:35 UTC
[redacted]	Clinical Approval	21-Feb-2022 17:03 UTC